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(54) Title: IMMUNOTOXINS COMPRISING GELONIN AND AN ANTIBODY

(57) Abstract

The present invention provides purified and isolated polynucleotides encoding Type I ribosome-inactivating proteins (RIPs) and analogs of the RIPs having a cysteine available for disulfide bonding to targeting molecules. Vectors comprising the polynucleotides and host cells transformed with the vectors are also provided. The RIPs and RIP analogs are particularly suited for use as components of cytotoxic therapeutic agents of the invention which include gene fusion products and immunoconjugates. Cytotoxic therapeutic agents or immunotoxins according to the present invention may be used to selectively eliminate any cell type to which the RIP component is targeted by the specific binding capacity of the second component of the agent, and are suited for treatment of diseases where the elimination of a particular cell type is a goal, such as autoimmune disease, cancer and graft-versus-host disease.

IMMUNOTOXINS COMPRISING GELONIN AND AN ANTIBODY

FIELD OF THE INVENTION

This application is a continuation-in-part of co-pending U.S. Patent Application Serial No. 08/064,691 filed May 12, 1993, which is a continuation-in-part of co-pending U.S. Patent Application Serial No. 07/988,430 filed December 9, 1992, which is in turn a continuation-in-part of pending U.S. Patent Application Serial No. 07/901,707 filed June 19, 1992, which in turn is a continuation-in-part of U.S. Patent Application Serial No. 07/787,567 filed November 4, 1991, now abandoned.

The present invention generally relates to materials useful as components of cytotoxic therapeutic agents. More particularly, the invention relates to ribosome-inactivating proteins, to analogs of ribosome-inactivating proteins, to polynucleotides encoding such proteins and analogs, some of which are specifically modified for conjugation to targeting molecules, and to gene fusions of polynucleotides encoding ribosome-inactivating proteins to polynucleotides encoding targeting molecules.

BACKGROUND

Ribosome-inactivating proteins (RIPs) comprise a class of proteins which is ubiquitous in higher plants. However, such proteins have also been isolated from bacteria. RIPs are potent inhibitors of eukaryotic protein synthesis. The N-glycosidic bond of a specific adenine base is hydrolytically cleaved by RIPs in a highly conserved loop region of the 28S rRNA of eukaryotic ribosomes thereby inactivating translation.

Plant RIPs have been divided into two types. Stirpe et al., *FEBS Lett.*, *195(1,2)*:1-8 (1986). Type I proteins each consist of a single peptide chain having ribosome-inactivating activity, while Type II proteins each consist of an A-chain, essentially equivalent to a Type I protein, disulfide-linked to a B-chain having cell-binding

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properties.   Gelonin,   dodecandrin,   tricosanthin,
tricokirin, bryodin, *Mirabilis* antiviral protein (MAP),
barley ribosome-inactivating protein (BRIP), pokeweed
antiviral proteins (PAPs), saporins, luffins, and momordins
5   are examples of Type I RIPs; whereas Ricin and abrin are
examples of Type II RIPs.

Amino acid sequence information is reported for
various ribosome-inactivating proteins. It appears that at
least the tertiary structure of RIP active sites is
10   conserved among Type I RIPs, bacterial RIPs, and A-chains
of Type II RIPs. In many cases, primary structure homology
is also found.   Ready *et al.*, *J. Biol. Chem.*,
259(24):15252-15256 (1984) and other reports suggest that
the two types of RIPs are evolutionarily related.

15   Type I plant ribosome-inactivating proteins may
be particularly suited for use as components of cytotoxic
therapeutic agents. A RIP may be conjugated to a targeting
agent which will deliver the RIP to a particular cell type
in vivo in order to selectively kill those cells.
20   Typically, the targeting agent (e.g., an antibody) is
linked to the toxin by a disulfide bond which is reduced *in
vivo* allowing the protein toxin to separate from the
delivery antibody and become active intracellularly.
Another strategy for producing targeted cytotoxic proteins
25   is to express a gene encoding a cytotoxic protein fused to
a gene encoding a targeting moiety. The resulting protein
product is composed of one or more polypeptides containing
the cytotoxic protein linked to, for example, at least one
chain of an antibody.

30   A variety of such gene fusions are discussed in
Pastan *et al.*, *Science*, 254:1173-1177 (1991). However,
these fusion proteins have been constructed with sequences
from diphtheria toxin or *Pseudomonas aeruginosa* exotoxin A,
both of which are ADP-ribosyltransferases of bacterial
35   origin.   These protein toxins are reported to intoxicate
cells and inhibit protein synthesis by mechanisms which
differ from those of the RIPs. Moreover, diphtheria toxin

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and exotoxin A are structurally different from, and show little amino acid sequence similarity with, RIPs. In general, fusion proteins made with diphtheria toxin or exotoxin A have been immunogenic and toxic in animals, and are produced intracellularly in relatively low yield. Another strategy for producing a cytotoxic agent is to express a gene encoding a RIP fused to a gene encoding a targeting moiety. The resulting protein product is a single polypeptide containing a RIP linked to, for example, at least one chain of an antibody.

Because some RIPs, such as the Type I RIP gelonin, are primarily available from scarce plant materials, it is desirable to clone the genes encoding the RIPs to enable recombinant production of the proteins. It is also desirable to develop analogs of the natural proteins which may be easily conjugated to targeting molecules while retaining their natural biological activity because most Type I RIPs have no natural sites (*i.e.* available cysteine residues) for conjugation to targeting agents. Alternatively, it is desirable to develop gene fusion products including Type I RIPs as a toxic moiety and antibody substances as a targeting moiety.

The present invention also provides novel humanized or human-engineered antibodies and methods for producing such antibodies which may be conjugated or fused to various toxins. Such conjugations or fusions are useful in the treatment of various disease states, including autoimmune diseases and cancer.

There are several reports relating to replacement of amino acids in a mouse antibody with amino acids normally occurring at the analogous position in the human form of the antibody. *See, e.g.*, Junghaus, *et al.*, *Cancer Res.*, *50*: 1495-1502 (1990) and other publications which describe genetically-engineered mouse/human chimeric antibodies. Also by genetic engineering techniques, the genetic information from murine hypervariable complementarity determining regions (hereinafter referred

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to as "CDRs") may be inserted in place of the DNA encoding the CDRs of a human monoclonal antibody to generate a construct encoding a human antibody with murine CDRs. *See, e.g.,* Jones, *et al., Nature, 321:* 522-525 (1986).

Protein structure analysis on such "CDR-grafted" antibodies may be used to "add back" murine residues in order to restore lost antigen-binding capability, as described in Queen, *et al, Proc. Natl. Acad. Sci. (USA), 86:*10029-10033 (1989); Co, *et al., Proc. Nat. Acad. Sci. (USA), 88:* 2869-2873 (1991). However, a frequent result of CDR-grafting is that the specific binding acitvity of the resulting humanized antibodies may be diminished or completely abolished.

As demonstrated by the foregoing, there exists a need in the art for cloned genes encoding Type I RIPs, for analogs of Type I RIPs which may be easily conjugated to targeting molecules, and for gene fusion products comprising Type I RIPs, and especially wherein such gene fusions also comprise an humanized antibody portion.

SUMMARY OF THE INVENTION

The present invention provides purified and isolated polynucleotides encoding Type I RIPs, Type I RIPs having a cysteine available for disulfide bonding to targeting molecules and fusion products comprising Type I RIPs. Vectors comprising the polynucleotides and host cells transformed with the vectors are also provided.

A purified and isolated polynucleotide encoding natural sequence gelonin (SEQ ID NO: 11), and a host cell including a vector encoding gelonin of the type deposited as ATCC Accession No. 68721 are provided. Further provided are a purified and isolated polynucleotide encoding natural sequence barley ribosome-inactivating protein and a purified and isolated polynucleotide encoding momordin II.

Some of the polynucleotides mentioned above encode fusion proteins of the present invention comprising gelonin (SEQ ID NO: 2) or another RIP and an antibody or a

fragment comprising an antigen-binding portion thereof. Several alternate forms of fusion proteins comprising gelonin are contemplated herein. For example, the fusion protein may contain a single RIP fused to a monovalent antibody binding moiety, such as a Fab or single chain antibody. Alternatively, multivalent forms of the fusion proteins may be made and may have greater activity than the monovalent forms. In preferred embodiments of the invention, gelonin may be fused to either the carboxy or the amino terminus of the antibody or antigen-binding portion of thereof. Also in a preferred embodiment of the invention, the antibody or fragment thereof comprising an antigen-binding portion may be an he3 antibody, an he3-Fab, an he3 Fd, single-chain antibody, or an he3 kappa fragment. The antibody or antigen-binding portion thereof may be fused to gelonin by means of a linker peptide, preferably a peptide segment of shiga-like toxin as shown in SEQ ID NO: 56 or a peptide segment of Rabbit muscle aldolase as shown in SEQ ID NO: 57 or a human muscle aldolase, an example of which is reported in Izzo, et al., *Eur. J. Biochem*, *174*: 569-578 (1988), incorporated by reference herein.

Analogs of a Type I plant RIP are defined herein as non-naturally occurring polypeptides that share the ribosome-inactivating activity of the natural protein but that differ in amino acid sequence from the natural type I RIP protein in some degree but less than they differ from the amino acid sequences of other Type I plant RIP. Preferred analogs according to the present invention are analogs of Type I plant RIPs each having a cysteine available for disulfide bonding located at a position in its amino acid sequence from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog. SEQ ID NO: 1 represents the amino acid sequence of ricin A-chain. Other preferred analogs according to the invention are Type I RIPs each having a cysteine available for disulfide bonding at a position in

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the analog that is on the surface of the protein in its natural conformation and that does not impair native folding or biological activity of the ribosome-inactivating protein. Analogs of bacterial RIPs are also contemplated

5   by the present invention.

The present invention provides an analog of a Type I ribosome-inactivating protein, which analog has a cysteine available for intermolecular disulfide bonding at an amino acid position corresponding to a position not

10   naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and which cysteine is located at a position in the amino acid sequence of the analog corresponding to position 259 in SEQ ID NO: 1 or at a position in the amino acid sequence in the analog

15   corresponding to a position from position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog.

An analog according to the present invention may be an analog of gelonin. In an analog of gelonin according to the present invention, the cysteine may be at a position in the analog from position 244 to the carboxyl terminal

20   position of the analog, more preferably at a position in the analog from position 247 to the carboxyl terminal position of the analog, and most preferably at position 244, at position 247, or at position 248 of the amino acid sequence of the analog. In these analogs, it is preferred

25   that the gelonin cysteine residues at positions 44 and 50 be replaced with non-cysteine residues such as alanine.

An analog according to the present invention may be an analog of barley ribosome-inactivating protein.

30   Preferably, a cysteine in such an analog is at a position in the analog from position 256 to the carboxyl terminal position, and more preferably the cysteine is at a position in the amino acid sequence of the analog from position 260 to the carboxyl terminal position of the analog. Most

35   preferably, in these regions, the cysteine is at position 256, at position 270, or at position 277 of the amino acid sequence of the analog.

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An analog according to the present invention may be an analog of momordin II.

Analogs according to the present invention may have a cysteine in the amino acid sequence of the analog at a position which corresponds to a position within one amino acid of position 259 of SEQ ID NO: 1. Such an analog may be an analog of gelonin, of barley ribosome-inactivating protein, or of momordin II.

The present invention also provides a polynucleotide encoding an analog of a Type I ribosome-inactivating protein, which analog has a cysteine available for intermolecular disulfide bonding at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein, and which cysteine is located at a position in the amino acid sequence of the analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog. The polynucleotide may encode an analog of gelonin, preferably an analog wherein the cysteine is at a position in the amino acid sequence of the analog from position 244 to the carboxyl terminal position of the analog, more preferably wherein the cysteine is at a position in the analog from position 247 to the carboxyl terminal position of the analog, and most preferably the cysteine is at position 244, at position 247 or at position 248 of the amino acid sequence of the analog. It is preferred that a polynucleotide according to the present invention encode a gelonin analog wherein the native gelonin cysteine residues at positions 44 and 50 are replaced with non-cysteine residues, such as alanine.

A polynucleotide according to the present invention may encode an analog of barley ribosome-inactivating protein, preferably an analog wherein the cysteine is at a position in the analog from position 256 to the carboxyl terminal position of the analog, more preferably wherein the cysteine is at a position in the

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analog from position 260 to the carboxyl terminal position
of the analog, and most preferably wherein the cysteine is
at position 256, at position 270 or at position 277 of the
amino acid sequence of the analog.

5   A polynucleotide according to the present
invention may encode an analog of mormordin II.

The present invention provides a vector including
a polynucleotide encoding an analog of a Type I ribosome-
inactivating protein, which analog has a cysteine available
10   for intermolecular disulfide bonding at a amino acid
position corresponding to a position not naturally
available for intermolecular disulfide bonding in the Type
I ribosome-inactivating protein and which cysteine is
located at a position in the amino acid sequence of the
15   analog from the position corresponding to position 251 in
SEQ ID NO: 1 to the carboxyl terminal position of the
analog.

The present invention further provides a host
cell including a DNA vector encoding an analog of a Type I
20   ribosome-inactivating protein, which analog has a cysteine
available for intermolecular disulfide bonding at an amino
acid position corresponding to a position not naturally
available for intermolecular disulfide bonding in the Type
I ribosome-inactivating protein and which cysteine is
25   located at a position in the amino acid sequence of the
analog from the position corresponding to position 251 in
SEQ ID NO: 1 to the carboxyl terminal position of the
analog.   In such a host cell the vector may encode an
analog of gelonin, especially an analog wherein the
30   cysteine is at position 247 of the amino acid sequence of
the analog, such as in the host cell deposited as ATCC
Accession No. 69009.

A host cell according to the present invention
may include a vector encoding barley ribosome-inactivating
35   protein, especially preferred is a host cell containing a
BRIP analog wherein the cysteine is at position 277, such
as in the host cell deposited on October 2, 1991 with the

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American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 as ATCC Accession No. 68722. Particularly preferred are prokaryotic host cells because such cells may be less sensitive to the action or RIPs as compared to eukaryotic cells.

The present invention also provides an agent toxic to a cell including an analog of a Type I ribosome-inactivating protein linked by a disulfide bond through a cysteine to a molecule which specifically binds to the cell, which cysteine is at an amino acid position in the analog corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and which cysteine is located in the amino acid sequence of the analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog. The agent may include an analog of gelonin, preferably an analog wherein the cysteine is at a position in the analog from position 247 to the carboxyl terminal position of the analog, and more preferably wherein the cysteine is at position 247 or 248 of the amino acid sequence of analog. An agent including an analog wherein the native gelonin cysteine residues at positions 44 and 50 are replaced with non-cysteine residues, such as alanine is preferred.

An agent according to the present invention may include an analog of barley ribosome-inactivating protein, preferably an analog wherein the cysteine is at a position in the analog from position 260 to the carboxyl terminal position of the analog, more preferably wherein the cysteine is at a position in the analog from position 270 to the carboxyl terminal position of the analog, and most preferably wherein the cysteine is at position 256, at position 270 or at position 277 of the amino acid sequence of the analog.

An agent according to the present invention may include an analog of momordin II.

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The present invention provides an agent wherein the Type I ribosome-inactivating protein is linked to an antibody, particularly to an H65 antibody or to an antibody fragment, more particularly to an antibody fragment

5   selected from the group consisting of chimeric and human engineered antibody fragments, and most particularly to a Fab antibody fragment, a Fab' antibody fragment or a $F(ab')_2$ antibody fragment. It is highly preferred that an agent according to the present invention include a chimeric or

10 human engineered antibody fragment selected from the group consisting of a Fab antibody fragment, a Fab' antibody fragment and a $F(ab')_2$ antibody fragment.

A method according to the present invention for preparing an analog of a Type I ribosome-inactivating

15 protein includes the step of expressing in a suitable host cell a polynucleotide encoding a Type I ribosome-inactivating fusion protein or type I RIP (especially gelonin) having a cysteine available for intermolecular disulfide bonding substituted (e.g., by site-directed

20 mutagenesis of the natural DNA sequence encoding the RIP or by chemical synthesis of a DNA sequence encoding the RIP analog) at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating

25 protein, which cysteine is located at a position in the amino acid sequence of the analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog.

A product according to the present invention may

30 be a product of a method including the step of expressing in a suitable host cell a polynucleotide encoding a Type I ribosome-inactivating protein having a cysteine available for intermolecular disulfide bonding substituted at an amino acid position corresponding to a position not

35 naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein, which cysteine is located at a position in the amino acid sequence of the

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analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog.

The present invention provides a method for preparing an agent toxic to a cell including the step of linking an analog of a Type I ribosome-inactivating protein through a cysteine to a molecule which specifically binds to the cell, which analog has the cysteine at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and which cysteine is located at a position in the amino acid sequence of the analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog.

According to the present invention, a method for treating a disease in which elimination of particular cells is a goal may include the step of administering to a patient having the disease a therapeutically effective amount of an agent toxic to the cells including a type I RIP (especially gelonin fused to or an analog of a Type I ribosome-inactivating protein linked through a cysteine to a molecule which specifically binds to the cell, the analog having the cysteine at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and the cysteine being located at a position in the amino acid sequence of the analog from the position corresponding to position 251 in SEQ ID NO: 1 to the carboxyl terminal position of the analog.

Useful target cells for immunotoxins of the present invention include, but are not limited to, cells which are pathogenic, such as cancer cells, autoimmune cells, and virally-infected cells. Such pathogenic cells may be targeted by antibodies or other targeting agents of the invention which are joined, either by genetic engineering techniques or by chemical cross-linking, to an

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RIP. Specifically useful targets include tumor-associated antigens (e.g., on cancer cells), cell differentiation markers (e.g., on autoimmune cells), parasite-specific antigens, bacteria-specific antigens, and virus-specific antigens.

The present invention also provides an analog of a Type I ribosome-inactivating protein, wherein the analog has a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation, and wherein the analog retains the ribosome-inactivating activity of the Type I ribosome-inactivating protein.

Such a fusion protein or an analog may be a fusion protein or an analog wherein the Type I ribosome inactivating protein is gelonin, and the analog is preferably an analog of gelonin wherein the cysteine is at position 10 of the amino acid sequence of the analog as encoded in a vector in a host cell deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 as ATCC Accession No. 69008 on June 9, 1992. Other such gelonin analogs include those wherein the cysteine is at a position 60, 103, 146, 184 or 215 in the amino acid sequence of the gelonin analog. It is preferred that the gelonin cysteine residues at positions 44 and 50 be replaced with non-cysteine residues such as alanine in these analogs.

The present invention further provides an analog of a Type I ribosome-inactivating protein wherein the analog includes only a single cysteine. Such an analog may be an analog of gelonin and is preferably an analog wherein the single cysteine is at position 10, position 44, position 50 or position 247 in the amino acid sequence of the analog, but the cysteine may be located at other positions defined by the invention as well.

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The present invention provides a polynucleotide encoding an analog of a Type I ribosome-inactivating protein, wherein the analog has a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation, and wherein the analog retains ribosome-inactivating activity of the Type I ribosome-inactivating protein.

According to the present invention, a method for preparing an analog of a Type I ribosome-inactivating protein may include the step of expressing in suitable host cell a polynucleotide encoding a Type I ribosome-inactivating protein having a cysteine available for intermolecular disulfide bonding substituted at an amino acid position corresponding to a position not naturally available for disulfide bonding in the Type I ribosome-inactivating protein, the cysteine is located at a position corresponding to an amino acid position on the surface of ricin A-chain in its natural conformation and which analog retains ribosome-inactivating activity of the Type I ribosome-inactivating protein.

The present invention provides an agent toxic to a cell including an analog of a Type I ribosome-inactivating protein linked by a disulfide bond through a cysteine to a molecule which specifically binds to the cell, wherein the analog has a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation, and wherein the analog retains ribosome-inactivating activity of the Type I ribosome-inactivating protein.

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A method according to the present invention for preparing an agent toxic to a cell may include the step of linking an analog of a Type I ribosome-inactivating protein through a cysteine to a molecule which specifically binds to the cell, which analog has a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation, and which analog retains ribosome-inactivating activity of the Type I ribosome-inactivating protein.

A method according to the present invention for treating a disease in which elimination of particular cells is a goal includes the step of administering to a patient having the disease a therapeutically effective amount of an agent toxic to the cells wherein the agent includes a type I RIP fused to or an analog of a Type I ribosome-inactivating protein linked by a disulfide bond through a cysteine to a molecule which specifically binds to the cell, which analog has a cysteine available for intermolecular disulfide bonding located at an amino acid position corresponding to a position not naturally available for intermolecular disulfide bonding in the Type I ribosome-inactivating protein and corresponding to a position on the surface of ricin A-chain in its natural conformation, and which analog retains ribosome-inactivating activity of the Type I ribosome-inactivating protein.

The RIP analogs of the invention are particularly suited for use as components of cytotoxic therapeutic agents. Cytotoxic agents according to the present invention may be used *in vivo* to selectively eliminate any cell type to which the RIP component is targeted by the specific binding capacity of the second component. To form cytotoxic agents, RIP analogs may be conjugated to

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monoclonal antibodies, including chimeric and CDR-grafted antibodies, and antibody domains/fragments (e.g., Fab, Fab', $F(ab')_2$, single chain antibodies, and Fv or single variable domains). Analogs of RIPs conjugated to monoclonal antibodies genetically engineered to include free cysteine residues are also within the scope of the present invention. Examples of Fab' and $F(ab')_2$ fragments useful in the present invention are described in co-pending, co-owned U.S. Patent Application Serial No. 07/714,175, filed June 14, 1991 and in International Publication No. WO 89/00999 published on February 9, 1989, which are incorporated by reference herein.

The RIP analogs of the invention may preferably be conjugated or fused to humanized or human engineered antibodies, such as the he3 antibody described herein. Such humanized antibodies may be constructed from mouse antibody variable domains, such as the mouse antibody H65 (SEQ ID NOS: 123 and 124). Specifically RIP analogs according to the present invention may be conjugated or fused to he3 human-engineered antibody light and heavy chain variable regions (SEQ ID NO: 125 and 126, respectively) or fragments thereof. A cell line producing an intact he3 immunoglobulin was deposited as ATCC Accession No. HB11206 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852.

RIPs according to the present invention may also be conjugated to targeting agents other than antibodies, for example, lectins which bind to cells having particular surface carbohydrates, hormones, lymphokines, growth factors or other polypeptides which bind specifically to cells having particular receptors. Immunoconjugates including RIPs may be described as immunotoxins. An immunotoxin may also consist of a fusion protein rather than an immunoconjugate.

The present invention provides gene fusions of an antigen-binding portion of an antibody (e.g., an antibody

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light chain or truncated heavy chain, or a single chain
antibody) or any targeting agent listed in the foregoing
paragraph, linked to a Type I RIP. Preferably, the
antigen-binding portion of an antibody or fragment thereof
5   comprises a single chain antibody, a Fab fragment, or a
F(ab')$_2$ fragment. Active antibodies generally have a
conserved three-dimensional folding pattern and it is
expected that any antibody which maintains that folding
pattern will retain binding specificity. Such antibodies
10   are expected to retain target enzymatic activity when
incorporated into a fusion protein according to the present
invention.

It is sometimes necessary that immunotoxins
comprising cytotoxic components, such as RIPs, be attached
15   to targeting agents via cleavable linkers (i.e.,
disulfides, acid-sensitive linkers, and the like) in order
to allow intracellular release of the cytotoxic component.
Such intracellular release allows the cytotoxic component
to function unhindered by possible negative kinetic or
20   steric effects of the attached antibody. Accordingly, gene
fusions of the present invention may comprise a RIP gene
fused, via a DNA segment encoding a linker protein as
described above, to either the 5' or the 3' end of a gene
encoding an antibody. If a linker is used, it preferably
25   encodes a polypeptide which contains two cysteine residues
participating in a disulfide bond and forming a loop which
includes a protease-sensitive amino acid sequence (e.g., a
segment of E. coli shiga-like toxin as in SEQ ID NO: 56) or
a segment which contains several cathepsin cleavage sites
30   (e.g., a segment of rabbit muscle aldolase as in SEQ ID NO:
57; a segment of human muscle aldolase; or a synthetic
peptide including a cathepsin cleavage sites such as in SEQ
ID NOs: 141 or 142). A linker comprising cathepsin
cleavage sites as exemplified herein comprises the C-
35   terminal 20 amino acids of RMA. However, that sequence
differs by only one amino acid from human muscle aldolase
and it is contemplated that muscle aldolase from human or

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other sources may be used as a linker in the manner described below. The Type I RIP portion of the fused genes preferably encodes gelonin, BRIP or momordin II. Also preferably, the antibody portion of the fused genes comprises sequences encoding one of the chains of an antibody Fab fragment (*i.e.*, kappa or Fd) and the fused gene is co-expressed in a host cell with the other Fab gene, or the antibody portion comprises sequences encoding a single chain antibody.

Alternatively, since fusion proteins of the present invention may be of low (approximately 55 kDa) molecular weight while maintaining full enzymatic activity, such fusions may be constructed without a linker and still possess cytotoxic activity. Such low-molecular weight fusions are not as susceptible to kinetic and steric hinderance as are the larger fusions, such as fusions involving IgG molecules. Therefore, cleavage of the RIP away from the fusion may not be necessary to facilitate activity of the RIP.

The present invention also provides a method for purifying a protein or immunotoxin comprising a ribosome-inactivating protein and a portion of an antibody including the steps of passing a solution containing the protein through an anion exchange column; applying the flow-through to a protein G column; and eluting the protein from the protein G column. The method may further comprise the steps of introducing the flow-through of the anion exchange column into a cation exchange column; exposing the cation exchange column to an eluent effective to elute said protein; and then applying the eluted protein to a protein G column, rather than applying the anion exchange column flow-through directly to a protein G column.

Immunotoxins according to the present invention, including immunoconjugates and fusion proteins (immunofusions), are suited for treatment of diseases where the elimination of a particular cell type is a goal, such as autoimmune disease, cancer, and graft-versus-host

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disease. The immunotoxins are also suited for use in causing immunosuppression and in treatment of infections by viruses such as the Human Immunodeficiency Virus.

Specifically illustrating polynucleotide sequences according to the present invention are the inserts in the plasmid pING3731 in *E. coli* MC1061 (designated strain G274) and in the plasmid pING3803 in *E. coli* E104 (designated strain G275), both deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland, on October 2, 1991, and assigned ATCC Accession Nos. 68721 and 68722, respectively. Additional polynucleotide sequences illustrating the invention are the inserts in the plasmid pING3746 in *E. coli* E104 (designated strain G277) and in the plasmid pING3737 in *E. coli* E104 (designated strain G276), which were both deposited with the ATCC on June 9, 1992, and were respectively assigned Accession Nos. 69008 and 69009. Still other polynucleotide sequences illustrating the invention are the inserts in the plasmid pING3747 in *E. coli* E104 (designated strain G278), in the plasmid pING3754 in *E. coli* E104 (designated strain G279), in the plasmid pING3758 in *E. coli* E104 (designated strain G280) and in the plasmid pING3759 in *E. coli* E104 (designated strain G281), which plasmids were all deposited with the ATCC on October 27, 1992 and were assigned ATCC Accession Nos. 69101, 69102, 69103 and 69104, respectively.

As noted above, RIPs may preferably be conjugated or fused to humanized or human-engineered antibodies, such as he3. Thus, the present invention also provides novel proteins comprising an humanized antibody variable domain which is specifically reactive with an human CD5 cell differentiation marker. Specifically, the present invention provides proteins comprising the he3 light and heavy chain variable regions as shown in SEQ ID NOS: 95 or 96, respectively. DNA encoding certain he3 proteins is shown in SEQ ID NOS: 67 and 68.

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In a preferred embodiment of the present invention, the protein comprising an humanized antibody variable region is an intact he3 immunoglobulin deposited as ATCC HB 11206.

5   Also in a preferred embodiment of the invention, the protein comprising an humanized antibody variable region is a Fab or F(ab')$_2$ or Fab fragment.

Proteins according to the present invention may be made by methods taught herein and in co-pending, co-
10   owned U.S. patent application no. 07/808,464 by Studnicka et al. incorporated by reference herein; and modified antibody variable domains made by such methods may be used in therapeutic administration to humans either alone or as part of an immunoconjugate as taught in co-owned, co-
15   pending U.S. Patent Application No. 07/787,567 by Better et al.

The present invention also provides methods for preparing a modified antibody variable domain useful in preparing immunotoxins and immunofusions by determining the
20   amino acids of a subject antibody variable domain which may be modified without diminishing the native affinity of the domain for antigen while reducing its immunogenicity with respect to a heterologous species. As used herein, the term "subject antibody variable domain" refers to the
25   antibody upon which determinations are made. The method includes the following steps: determining the amino acid sequence of a subject light chain and a subject heavy chain of a subject antibody variable domain to be modified; aligning by homology the subject light and heavy chains
30   with a plurality of human light and heavy chain amino acid sequences; identifying the amino acids in the subject light and heavy chain sequences which are least likely to diminish the native affinity of the subject variable domain for antigen while, at the same time, reducing its
35   immunogenicity by selecting each amino acid which is not in an interface region of the subject antibody variable domain and which is not in a complementarity-determining region or

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in an antigen-binding region of the subject antibody variable domain, but which amino acid is in a position exposed to a solvent containing the antibody; changing each residue identified above which aligns with a highly or a

5   moderately conserved residue in the plurality of human light and heavy chain amino acid sequences if said identified amino acid is different from the amino acid in the plurality.

Another group of sequences, such as those in

10   Figures 1A and 1B may be used to determine an alignment from which the skilled artisan may determine appropriate changes to make.

The present invention provides a further method wherein the plurality of human light and heavy chain amino

15   acid sequences is selected from the human consensus sequences in Figures 10A and 10B.

In general, human engineering according to the above methods may be used to treat various diseases against which monoclonal antibodies generally may be effective.

20   However, humanized antibodies possess the additional advantage of reducing the immunogenic response in the treated patient.

Additional aspects and applications of the present invention will become apparent to the skilled

25   artisan upon consideration of the detailed description of the invention which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (RTA) (SEQ ID NO:

30   1) with the amino acid sequence of the Type I ribosome-inactivating protein gelonin (SEQ ID NO: 2), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 2 is a computer-generated alignment of the

35   amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-

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inactivating protein BRIP (SEQ ID NO: 3), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 3 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-inactivating protein momordin II (MOMOII) (SEQ ID NO: 4), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 4 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-inactivating protein luffin (SEQ ID NO: 5), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 5 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-inactivating protein αtrichosanthin (TRICHO) (SEQ ID NO: 6), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 6 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-inactivating protein momordin I (MOMOI) (SEQ ID NO: 7), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 7 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-inactivating protein *Mirabilis* anti-viral protein (MAP) (SEQ ID NO: 8), wherein starred positions indicate amino acids invariant among the ricin A-chain and the Type I RIPs;

FIG. 8 is a computer-generated alignment of the amino acid sequence of the ricin A-chain (SEQ ID NO: 1) with the amino acid sequence of the Type I ribosome-

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inactivating protein pokeweed antiviral protein from seeds
(PAPS) (SEQ ID NO: 9), wherein starred positions indicate
amino acids invariant among the ricin A-chain and the Type
I RIPs;

5   FIG. 9 is a computer-generated alignment of the
amino acid sequence of the ricin A-chain (SEQ ID NO: 1)
with the amino acid sequence of the Type I ribosome-
inactivating protein saporin 6 (SAP6) (SEQ ID NO: 10),
wherein starred positions indicate amino acids invariant
10   among the ricin A-chain and the Type I RIPs;

         FIGs. 10A and 10B are alignments of the consensus
amino acid sequences for the subgroups of light [hK1 (human
kappa light chain subgroup 1), kH3 (human kappa light chain
subgroup 3), hK2 (human kappa light chain subgroup 2), hL1
15   (human lambda light chain subgroup 1), hL2 (human lambda
light chain subgoup 2), hL3 (human lambda light chain
subgroup 3), hL6 (human lambda light chain subgroup 6), hK4
(human kappa light chain subgroup 4), hL4 (human lambda
light chain subgroup 4) and hL5 (human lambda light chain
20   subgroup 5] and heavy chains [hH3 (human heavy chain
subgroup 3), hH1 (human heavy chain subgroup 1) and hH2
(human heavy chain subgroup 2)], respectively, of human
antibody variable domains;

         FIG. 11 sets out the nucleotide sequences of the
25   oligonucleotides utilized in the construction of the genes
encoding modified V/J-regions of the light and heavy chains
of the H65 mouse monoclonal antibody variable domain; and

         FIGs. 12A and 12B are alignments of human light
chain consensus hK1 and heavy chain consensus hH1 with the
30   light and heavy chain sequences, respectively, of the
variable domain of human antibody EU, human antibody TAC,
human antibody TAC modified according to the present
invention (prop) and human antibody TAC modified according
to a different method (Que).

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DETAILED DESCRIPTION

Nucleotide sequences of genes encoding three plant Type I RIPs and expression vectors containing the genes are provided by the present invention. A first plant RIP, gelonin, is produced by seeds of *Gelonium multiflorum*, a plant of the Euphorbiaceae family native to the tropical forests of eastern Asia, while a second plant RIP, BRIP, is synthesized by the common cereal grain barley. Momordin II, a third plant RIP, is produced in *Momordica balsamina* seeds. Analogs of BRIP are also provided by the present invention. The analogs were genetically engineered to include a cysteine free to participate in a intermolecular disulfide bond and were conjugated to antibody molecules without non-specific chemical derivatization of the RIP with crosslinking agents.

Type I RIP analogs of the present invention offer distinct advantages over the natural proteins for use as components of immunotoxins. Chemical treatment to introduce free sulfhydryl groups in the natural proteins lacking free cysteines typically involves the non-selective modification of amino acid side chains. This non-selectivity often results in antibodies conjugated to different sites on different RIP molecules (*i.e.*, a heterogeneous population of conjugates) and also in a decrease in RIP activity if antibodies are conjugated in or near important regions of the RIP (*e.g.*, the active site or regions involved in translocation across cell membranes). In contrast, RIP analogs according to the present invention may be conjugated to a single antibody through a disulfide bond to a specific residue of the analog resulting in reduced batch to batch variation of the immunoconjugates and, in some cases, immunoconjugates with enhanced properties (*e.g.*, greater cytotoxicity or solubility).

Type I plant RIPs, as well as bacterial RIPs such as shiga and shiga-like toxin A-chains, are homologous to the ricin A-chain and are useful in the present invention.

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Type I RIPs may be defined and sites for substitution of a cysteine in a RIP may be identified by comparing the primary amino acid sequence of the RIP to the natural ricin A-chain amino acid sequence, the tertiary structure of which has been described in Katzin *et al.*, *Proteins*, *10*:251-259 (1991), which is incorporated by reference herein.

Amino acid sequence alignment defines Type I RIPs in that the ricin A-chain and the Type I plant RIPs have nine invariant amino acids in common. Based on the ricin sequence the invariant amino acids are tyrosine$_{21}$, arginine$_{29}$, tyrosine$_{80}$, tyrosine$_{123}$, leucine$_{144}$, glutamic acid$_{177}$, alanine$_{178}$, arginine$_{180}$, and tryptophan$_{211}$. The ricin A-chain may be used as a model for the three-dimensional structure of Type I RIPs. A protein lacking a cysteine available for conjugation while having ribosome-inactivating activity and having the nine invariant amino acids when its primary sequence is compared to the primary sequence of the ricin A-chain [according to the alignment algorithm of Myers *et al.*, *CABIOS COMMUNICATIONS, 4(1)*:11-17 (1988), implemented by the PC/GENE program PALIGN (Intelligenetics, Inc., Mountain View, California) and utilizing the Dayhoff Mutation Data Matrix (MDM-78) as described in Schwartz *et al.*, pp. 353-358 in *Atlas of Protein Sequence and Structure*, 5 Supp. 3, National Biomedical Research Foundation, Washington, D.C. (1978)] is defined as a Type I RIP herein and is expected to be useful in the present invention. "Corresponding" refers herein to amino acid positions which align when two amino acid sequences are compared by the strategy of Myers *et al.*, *supra*.

The primary amino acid sequences of the Type I RIPs:gelonin, BRIP, momordin II, luffin [see Islam *et al.*, *Agricultural Biological Chem.*, *54(5)*:1343-1345 (199)], αtrichosanthin [see Chow *et al.*, *J. Biol. Chem.*, *265*:8670-8674 (1990)], momordin I [see Ho *et al.*, *BBA*, *1088*:311-314 (1991)], *Mirabilis* anti-viral protein [see Habuka *et al.*,

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J. Biol. Chem., 264(12):6629-6637 (1989)], pokeweed
antiviral protein isolated from seeds [see Kung et al.,
Agric. Biol. Chem., 54(12):3301-3318 (1990)] and saporin
[see Benatti et al., Eur. J. Biochem., 183:465-470 (1989)]
5   are individually aligned with the primary sequence of the
ricin A-chain [see Halling et al., Nucleic Acids Res.,
13:8019-8033 (1985)] in FIGs 1-9, respectively, according
to the algorithm of Myers et al., supra, as specified
above.

10   FIGS. 1-9 may be utilized to predict the amino
acid positions of the Type I RIPs where cysteine residues
may be substituted. Preferred amino acids for cysteine
substitution are on the surface of the molecule and include
any solvent accessible amino acids which will not interfere
15   with proper folding of the protein if replaced with a
cysteine. A region of the ricin A-chain comprising such
amino acids is the carboxyl terminal region. Amino acids
that should be avoided for replacement are those critical
for proper protein folding, such as proline, and those that
20   are solvent inaccessible. Also to be avoided are the nine
amino acids invariant among RIPs, and the amino acids in or
near regions comprising the active site of ricin A-chain as
depicted in Figure 6 of Katzin et al., supra.

            Therefore, a preferred region of substitution for
25   Type I RIPs is their carboxyl terminal region which is
solvent accessible and corresponds to the carboxyl terminal
region where Type II RIP A-chains and B-chains are
naturally linked by a disulfide bond. As shown in the
examples, a cysteine may be substituted in positions in the
30   amino acid sequence of a Type I RIP from the position
corresponding to position 251 in SEQ ID NO: 1 to the
carboxyl terminal position of said Type I RIP, resulting in
RIP analogs which retain enzymatic activity and gain
disulfide cross-linking capability. One preferred cysteine
35   substitution position is near the position which
corresponds to the cysteine at position 259 in the ricin A-
chain.

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For purposes of the present invention, immunotoxins comprise a class of compounds of which toxin-antibody fusions and immunoconjugates are examples. Immunotoxins are particularly suited for use in treatment of human autoimmune diseases and in the treatment of diseases in which depletion of a particular cell type is a goal, such as cancer. For example, treatment of autoimmune diseases with immunotoxins is described in International Publication No. WO89/06968 published August 10, 1989, which is incorporated by reference herein.

In any treatment regimen, the immunotoxins may be administered to a patient either singly or in a cocktail containing two or more immunotoxins, other therapeutic agents, compositions, or the like, including, but not limited to, immunosuppressive agents, tolerance-inducing agents, potentiators and side-effect relieving agents. Particularly preferred are immunosuppressive agents useful in suppressing allergic reactions of a host. Preferred immunosuppressive agents include prednisone, prednisolone, DECADRON (Merck, Sharp & Dohme, West Point, Pennsylvania), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Preferred potentiators include monensin, ammonium chloride, perhexiline, verapamil, amantadine and chloroquine. All of these agents are administered in generally-accepted efficacious dose ranges such as those disclosed in the *Physician's Desk Reference*, 41st Ed., Publisher Edward R. Barnhart, New Jersey (1987). Patent Cooperation Treaty (PCT) patent application WO 89/069767 published on August 10, 1989, discloses administration of an immunotoxin as an immunosuppressive agent and is incorporated by reference herein.

Immunotoxins of the present invention may be formulated into either an injectable or topical preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for intramuscular or intravenous administration. The

formulations containing therapeutically-effective amounts of immunotoxins are either sterile liquid solutions, liquid suspensions, or lyophilized versions, and optionally contain stabilizers or excipients. Lyophilized compositions are reconstituted with suitable diluents, e.g., water for injection, saline, 0.3% glycine and the like, at a level of about from 0.01 mg/kg of host body weight to 10 mg/kg where the biological activity is less than or equal to 20 ng/ml when measured in a reticulocyte lysate assay. Typically, the pharmaceutical compositions containing immunotoxins of the present invention are administered in a therapeutically effective dose in a range of from about 0.01 mg/kg to about 5 mg/kg of the patient. A preferred, therapeutically effective dose of the pharmaceutical composition containing immunotoxins of the invention is in a range of from about 0.01 mg/kg to about 0.5 mg/kg body weight of the patient administered over several days to two weeks by daily intravenous infusion, each given over a one hour period, in a sequential patient dose-escalation regimen.

Immunotoxin compositions according to the invention may be formulated into topical preparations for local therapy by including a therapeutically effective concentration of immunotoxin in a dermatological vehicle. The amount of immunotoxin to be administered, and the immunotoxin concentration in the topical formulations, depend upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the immunotoxin in the formulation. Thus, a physician knows to employ the appropriate preparation containing the appropriate concentration of immunotoxin in the formulation, as well as the appropriate amount of formulation to administer depending upon clinical experience with the patient in question or with similar patients. The concentration of immunotoxin for topical formulations is in the range of greater than from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of

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immunotoxin for topical formulations is in the range of greater than from about 1 mg/ml to about 20 mg/ml. Solid dispersions of immunotoxins according to the invention, as well as solubilized preparations, may be used. Thus, the precise concentration to be used in the vehicle is subject to modest experimental manipulation in order to optimize the therapeutic response. For example, greater than about 10 mg immunotoxin/100 grams of vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to gels, are oil-in-water or water-in-oil emulsions using mineral oils, petroleum and the like.

Immunotoxins according to the invention may be optionally administered topically by the use of a transdermal therapeutic system [Barry, *Dermatological Formulations*, p. 181 (1983) and literature cited therein]. While such topical delivery systems may be designed for transdermal administration of low molecular weight drugs, they are capable of percutaneous delivery. Further, such systems may be readily adapted to administration of immunotoxin or derivatives thereof and associated therapeutic proteins by appropriate selection of the rate-controlling microporous membrane.

Topical preparations of immunotoxin either for systemic or local delivery may be employed and may contain excipients as described above for parenteral administration and other excipients used in a topical preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Pharmacologically-acceptable buffers may be used, e.g., Tris or phosphate buffers. The topical formulations may also optionally include one or more agents variously termed enhancers, surfactants, accelerants, adsorption promoters or penetration enhancers, such as an agent for enhancing percutaneous penetration of the immunotoxin or other agents. Such agents should desirably possess some or all of the following features as would be known to the

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ordinarily skilled artisan: pharmacological inertness, non-promotive of body fluid or electrolyte loss, compatible with immunotoxin (non-inactivating), and capable of formulation into creams, gels or other topical delivery
5   systems as desired.

Immunotoxins according to the present invention may also be administered by aerosol to achieve localized delivery to the lungs. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid
10   particles containing immunotoxin. Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of immunotoxin together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary depending upon the
15   requirements for the particular immunotoxin, but typically include: nonionic surfactants (Tweens, Pluronics, or polyethylene glycol); innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin; amino acids such as glycine; and buffers, salts, sugars or sugar
20   alcohols. The formulations may also include mucolytic agents as well as bronchodilating agents. The formulations are sterile. Aerosols generally are prepared from isotonic solutions. The particles optionally include normal lung surfactants.

25   Alternatively, immunotoxins of the invention may be administered orally by delivery systems such as proteinoid encapsulation as described by Steiner, *et al.*, U.S. Patent No. 4,925,673, incorporated by reference herein. Typically, a therapeutically-effective oral dose
30   of an immunotoxin according to the invention is in the range from about 0.05 mg/kg body weight to about 50 mg/kg body weight per day. A preferred effective dose is in the range from about 0.05 mg/kg body weight to about 5 mg/kg body weight per day.

35   Immunotoxins according to the present invention may be administered systemically, rather than topically, by injection intramuscularly, subcutaneously, intrathecally or

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intraperitoneally or into vascular spaces, particularly
into the joints, e.g., intraarticular injection at a dosage
of greater than about 1 μg/cc joint fluid/day. The dose
will be dependent upon the properties of the specific
5   immunotoxin employed, e.g., its activity and biological
half-life, the concentration of immunotoxin in the
formulation, the site and rate of dosage, the clinical
tolerance of the patient involved, the disease afflicting
the patient and the like, as is well within the skill of
10 the physician.

        The immunotoxins of the present invention may be
administered in solution. The pH of the solution should be
in the range of pH 5 to 9.5, preferably pH 6.5 to 7.5. The
immunotoxin or derivatives thereof should be in a solution
15 having a suitable pharmaceutically-acceptable buffer such
as   phosphate,   Tris(hydroxymethyl)aminomethane-HCl   or
citrate and the like. Buffer concentrations should be in
the range of 1 to 100 mM. The immunotoxin solution may
also contain a salt, such as sodium chloride or potassium
20 chloride in a concentration of 50 to 150 mM. An effective
amount of a stabilizing agent such as an albumin, a
globulin, a gelatin, a protamine or a salt of protamine may
also be included, and may be added to a solution containing
immunotoxin or to the composition from which the solution
25 is prepared.

        Systemic administration of immunotoxin may be
made daily and is generally by intramuscular injection,
although   intravascular   infusion   is   acceptable.
Administration may also be intranasal or by other
30 nonparenteral routes. Immunotoxins of the present
invention may also be administered via microspheres,
liposomes or other microparticulate delivery systems placed
in certain tissues including blood. Topical preparations
are applied daily directly to the skin or mucosa and are
35 then preferably occluded, i.e., protected by overlaying a
bandage, polyolefin film or other barrier impermeable to
the topical preparation.

The following Examples are illustrative of practice of the invention but are not to be construed as limiting the invention. The present application is broadly organized as follows. The first portion of the application broadly teaches the preparation, expression and properties of an exemplary RIP, gelonin. A second portion of the application teaches the preparation of human-engineered antibodies. A third portion of the application teaches the construction and properties of immunoconjugates, comprising an RIP and an antibody or fragment thereof comprising an antigen-binding portion. A forth portion of the application relates to the preparation and properties of immunofusion proteins comprising an RIP and an antibody or fragment thereof comprising an antigen-binding portion. A fifth portion of the application teaches the preparation and properties of the RIP Barley ribosome-inactivating protein and a final aspect of the invention provides the preparation and properties of the RIP momordin.

Specifically, Example 1 relates to the preparation of the RIP gelonin. Construction of expression vector, comprising the gelonin gene, including expression and purification of gelonin, is taught in Example 2. The assembly of gelonin genes with cysteine residues available for conjugation is taught in Example 3 and Example 4 provides results of a reticulocyte lysate assay performed on gelonin.

Example 5 teaches the construction of human-engineered antibodies for use in immunotoxins of the invention and Example 6 demonstrates transfection of he3 genes, expression of those genes, and purification of the products thereof.

Example 7 next teaches the preparation of gelonin immunoconjugates. The procedures and results of whole cell kill assays are next presented in Example 8. Various properties of gelonin immunoconjugates are taught in Example 9 and Examples 10 and 11 teach the pharmacokinetics of two types of immunoconjugates. Examples 12 and 13 teach

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the immunogenicity of immunoconjugates of the invention and the *in vivo* efficacy of those immunoconjugates, respectively.

5   The construction of genes encoding gelonin immunofusions is taught in Examples 14, 15, 16, 17 and 18. Example 19 teaches alternative cathepsin cleavable linkers for use in the immunofusions of the invention. The expression and purification of various genes encoding immunoconjugates are presented in Example 20 and their
10   activity properties are presented in Example 21.

The construction of genes encoding the RIP, BRIP, and its expression and properties are taught in Examples 22, 23, and 24.

Finally, construction of genes encoding momordin
15   and properties of momordin on expression are taught in Example 25.

Example 1

Preparation Of Gelonin

The cloning of the gelonin gene according to the
20   present invention obviates the requirement of purifying the RIP gene product from its relatively scarce natural source, *G. multiflorum* seeds. Cloning also allows development of gelonin analogs which may be conjugated to antibodies without prior chemical derivatization and also allows
25   development of gelonin gene fusion products.

A.   Preparation Of RNA From *G. Multiflorum* Seeds

Total RNA was prepared from *Gelonium* seeds (Dr. Michael Rosenblum, M.D. Anderson Cancer Center, Houston, Texas) by a modification of the procedure for preparation
30   of plant RNA described in Ausubel *et al., eds., Current Protocols in Molecular Biology*, Wiley & Sons, 1989. Briefly, 4.0 grams of seeds were ground to a fine powder in a pre-cooled (-70°C) mortar and pestle with liquid N_2. The powder was added to 25 ml Grinding buffer (0.18M Tris,
35   0.09M LiCl, 4.5mM EDTA, 1% SDS, pH 8.2) along with 8.5 ml

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of phenol equilibrated with TLE (0.2M Tris, 0.1M LiCl, 5mM EDTA pH8.2). The mixture was homogenized using a Polytron PT-1035 (#5 setting). 8.5 ml of chloroform was added, mixed and incubated at 50°C for 20 minutes. The mixture

5   was centrifuged at 3000 g for 20 minutes in a rotor precooled to 4°C and the aqueous phase was transferred to a new tube. 8.5 ml of phenol was added followed by 8.5 ml of chloroform and the mixture was recentrifuged. This extraction was repeated 3 times. The RNA in the aqueous

10   : phase was then precipitated by adding 1/3 volume 8M LiCl, and incubated at 4°C for 16 hours. Next, the RNA was pelleted by centrifugation for 20 minutes at 4°C. The pellet was washed with 5 ml of 2M LiCl, recentrifuged and resuspended in 2 ml of water. The RNA was precipitated by

15   addition of NaOAc to 0.3M and 2 volumes of ethanol. The RNA was stored in 70% ethanol at -70°C.

     B.   cDNA Preparation

          cDNA was prepared from total *Gelonium* RNA by two methods. The first method involved making a cDNA library

20   in the bacterial expression plasmid pcDNAII using the Librarian II cDNA Library Construction System kit (Invitrogen). Approximately 5 μg of total RNA was converted to first strand cDNA with a 1:1 mixture of random primers and oligo-dT. Second strand synthesis with DNA

25   polymerase I was performed as described by the system manufacturer. Double stranded cDNA was ligated to *BstX1* linkers and size fractionated. Pieces larger than about 500 bp were ligated into the expression vector provided in the kit. Individual vectors were introduced into *E. coli*

30   either by transformation into high-efficiency competent cells or by electroporation into electrocompetent cells. Electroporation was performed with a BTX100 unit (BTX, San Diego, CA) in 0.56μ Flatpack cells as recommended by BTX based on the method of Dower *et al.*, *Nucleic Acids Res.*,

35   *16*:6127-6145 (1988), at a voltage amplitude of 850 V and a

pulse length of 5 mS. The resulting library consisted of approximately 150,000 colonies.

The second method involved generating cDNA using the RNA-PCR kit sold by Perkin-Elmer-Cetus. About 100 ng of total Gelonium RNA was used as template for cDNA synthesis.

C. Determination Of The Gelonin Protein Sequence

The partial sequence of the native gelonin protein was determined by direct amino acid sequence analysis using automated Edman degradation as recommended by the manufacturer using an Applied Biosystems model 470A protein sequencer. Proteolytic peptide fragments of gelonin (isolated from the same batch of seeds as the total RNA) were sequenced.

D. Cloning Of The Gelonin Gene

Three overlapping gelonin cDNA fragments were cloned and a composite gelonin gene was assembled from the three fragments.

1. Cloning Of The Fragment Encoding The Middle Amino Acids Of Gelonin In Vector pING3823

Degenerate DNA primers based on the gelonin partial amino acid sequences were used to PCR-amplify segments of the cDNA generated with Perkin-Elmer-Cetus kit. Six primers were designed based on regions of the gelonin amino acid sequence where degeneracy of the primers could be minimized. Appropriate pairs of primers were tested for amplification of a gelonin gene fragment. Products of the expected DNA size were identified as ethidium bromide-stained DNA bands on agarose gels that DNA was treated with T4 DNA polymerase and then purified from an agarose gel. Only the primer pair consisting of primers designated gelo-7 and gelo-5 yielded a relatively pure product of the expected size. The sequences of degenerate primers gelo-7 and gelo-5 are set out below using IUPAC nucleotide symbols.

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Gelo-7 (SEQ ID NO: 14)

5' TTYAARGAYGCNCCNGAYGCNGCNTAYGARGG 3'

Gelo-5 (SEQ ID NO: 15)

3' TTYTTYATRATRCANTGNCGNCANCTRGTYCA 5'

Primer gelo-7 corresponds to amino acids 87-97 of gelonin while primer gelo-5 corresponds to amino acids 226-236. The blunt-ended DNA fragment (corresponding to amino acids 87 to 236 of gelonin) generated with primers gelo-7 and gelo-5 was cloned into pUC18 (BRL, Gaithersburg, Maryland). The DNA sequence of the insert was determined, and the deduced amino acid sequence based on the resulting DNA sequence matched the experimentally determined gelonin amino acid sequence. The clone containing this gelonin segment was denoted pING3726.

The insert of clone pING3726 was labeled with ^{32}P and used as a probe to screen the 150,000-member Gelonium cDNA library. Only one clone hybridized to the library plated in duplicate. This clone was purified from the library and its DNA sequence was determined. The clone contains a fragment encoding 185 of the 270 amino acids of gelonin (residues 25-209) and is denoted pING3823.

2.   Cloning Of The Fragment Encoding
The N-Terminal Amino Acids Of Gelonin

Based on the sequence determined for the gelonin gene segment in pING3726, exact oligonucleotide primers were designed as PCR amplification primers to be used in conjunction with a degenerate primer to amplify a 5' gelonin gene fragment and with a nonspecific primer to amplify a 3' gelonin gene fragment. cDNA generated using the Perkin-Elmer-Cetus RNA-PCR kit was amplified.

To amplify the 5'-end of the gelonin gene, PCR amplification with a degenerate primer gelo-1 and an exact primer gelo-10 was performed. The sequences of the primers are set out below.

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Gelo-1 (SEQ ID NO: 16)

5' GGNYTNGAYACNGTNWSNTTYWSNACNAARGG 3'

Gelo-10 (SEQ ID NO: 17)

3' TGTCTGAACCCGTAACTTGGTAA 5'

5   Primer gelo-1 corresponds to amino acids 1-11 of the
gelonin gene while primer gelo-10 corresponds to amino
acids 126-133. The product from the reaction was re-
amplified with gelo-1 (SEQ ID NO: 16) and gelo-11 (an exact
primer comprising sequences encoding amino acids 119-125 of
10   gelonin) to confer specificity to the reaction product.
The sequence of primer gelo-11 is listed below.

Gelo-11 (SEQ ID NO: 18)

3' CACTCTTCCGTATATCTCTCTGT 5'

Hybridization with an internal probe confirmed that the
15   desired specific gelonin DNA fragment was amplified. That
fragment was cloned into pUC18 and the vector generated was
designated pING3727. The fragment was sequenced, revealing
that the region of the fragment (the first 27 nucleotides)
corresponding to part of the degenerate primer gelo-1 could
20   not be translated to yield the amino acid sequence upon
which primer gelo-1 was originally based. This was not
unexpected considering the degeneracy of the primer. The
fragment was reamplified from the Gelonium cDNA with exact
primers gelo-11 (SEQ ID NO: 18) and gelo-5' (which extends
25   upstream of the 5' end of the gelonin gene in addition to
encoding the first 16 amino acids of gelonin). The
sequence of primer gelo-5' is set out below.

Gelo-5' (SEQ ID NO: 19)

5' TCAACCCGGGCTAGATACCGTGTCAT

30   TCTCAACCAAAGGTGCCACTTATATTA 3'

The resulting DNA fragment encodes the first 125 amino
acids of gelonin. While the majority of the sequence is
identical to the natural gelonin gene, the first 32
nucleotides of the DNA fragment may be different. For the
35   purposes of this application this N-terminal fragment is
referred to as fragment GEL1-125.

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3. Cloning Of The Fragment Encoding The C-Terminal Amino Acids Of Gelonin

To amplify the 3'-end of the gelonin gene as well as 3' untranslated sequences, PCR amplification with exact primers gelo-9 and XE-dT was performed. The sequence of each of the primers is set out below.

    Gelo-9 (SEQ ID NO: 20)
    5' CTTCATTTTGGCGGCACGTATCC 3'
    XE-dT (SEQ ID NO: 21)
    3' TTTTTTTTTTTTTTTTTTTTTAG
    GGTGCATTCGAACGTCGGAGCTC 5'

Primer gelo-9 corresponds to amino acids 107-113 of gelonin. Primer XE-dT consists of a 3' oligo-dT portion and a 5' portion containing the restriction sites HindIII and XhoI, and will prime any poly A-containing cDNA. The reaction product was reamplified with exact primers gelo-8 and XE. The sequences of primers gelo-8 and XE are set out below.

    Gelo-8 (SEQ ID NO: 22)
    5' CTCGCTGGAAGGTGAGAA 3'
    XE (SEQ ID NO: 23)
    3' AGGGTGCATTCGAACGTCGGAGCTC 5'

Primer gelo-8 consists of sequences encoding amino acids 115-120 of gelonin while the primer XE corresponds to the 5' portion of the XE-dT primer which contains HindIII and XhoI restriction sites. Hybridization with internal probes confirmed that the desired gelonin gene fragment was amplified. That fragment was then cloned into pUC18 by two different methods. First, it was cloned as a blunt-ended fragment into the SmaI site of pUC18 (the resulting vector was designated pING3728) and, second, it was cloned as an EcoRI to HindIII fragment into pUC18 (this vector was designated pING3729). Both vector inserts were sequenced. The insert of pING3728 encodes amino acids 114-270 of gelonin, while the insert of pING3729 encodes amino acids 184-270 of gelonin plus other 3' sequences.

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4. Assembly Of The Overlapping Gelonin DNA Fragments Into A Composite Gelonin Gene

To reassemble the C-terminal two-thirds of the gelonin gene, vector pING3729 was cut with *SspI* (one *SspI* site is located within the vector and the second is located about 80 bp downstream of the termination codon of the insert in the vector) and an *XhoI* linker (8 bp, New England Biolabs) was ligated to the resulting free ends. The DNA was then cut with *XhoI* and *EcoRI*, and the 350 bp fragment generated, encoding amino acids 185-270 of gelonin, was isolated. This 350 bp fragment was ligated adjacent to a *NcoI* to *EcoRI* fragment from pING3823 encoding amino acids 37-185 of gelonin in a intermediate vector denoted pING3730, thus reassembling the terminal 87% of the gelonin gene (amino acids 37-270).

Next, fragment GEL1-125 was cut with *SmaI* and *NcoI*, resulting in a fragment encoding amino acids 1-36 of gelonin which was ligated along with the *NcoI* to *XhoI* fragment of pING3730 into the vector pIC100. [pIC100 is identical to pING1500 described in Better, *et al., Science,* *240*:1041-1043 (1988), incorporated by reference herein], except that it lacks 37 bp upstream of the *pelB* leader sequence. The 37 bp were eliminated by digestion of pING1500 with *SphI* and *EcoRI*, treatment with T4 polymerase, and religation of the vector. This manipulation regenerated an *EcoRI* site in the vector while eliminating other undesirable restriction sites.] Before ligation, the vector pIC100 had previously been digested with *SstI*, treated with T4 polymerase, and cut with *XhoI*. The ligation generated a new vector containing a complete gelonin gene which was designated plasmid pING3731 and deposited with The American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852 on October 2, 1991 as Accession No. 68721. The complete DNA sequence of the gelonin gene is set out in SEQ ID NO: 11.

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Example 2

A.   Construction Of Expression
Vectors Containing The Gelonin Gene

A first *E. coli* expression vector was constructed containing the gelonin gene linked to the *Erwinia carotovora* pelB leader sequence, and to the *Salmonella typhimurium* araB promoter. A basic vector containing the araB promoter is described in co-owned U.S. Patent No. 5,028,530 issued July 2, 1991 which is incorporated by reference herein. The vector containing the araB promoter was cut with *EcoRI* and *XhoI*. Two DNA fragments were then ligated in tandem immediately downstream of the promoter. The fragment ligated adjacent to the promoter was a 131 bp fragment derived from *SstI* digestion, T4 polymerase treatment and digestion with *EcoRI* of the pIC100 vector which includes the leader sequence of the *E. carotovora* pelB gene. The translated leader sequence is a signal for secretion of the respective protein through the cytoplasmic membrane. The fragment ligated downstream of the leader sequence was a *SmaI* to *XhoI* fragment from pING3731 which contains the complete gelonin gene. Thus, the expression vector contains the gelonin gene linked to the pelB leader sequence and the araB promoter. This plasmid is designated pING3733.

A second expression vector may be constructed that is identical to the first except that the gelonin gene sequences encoding the nineteen C-terminal amino acids of gelonin are not included. The cDNA sequence of the gelonin gene predicted a 19 residue C-terminal segment that was not detected in any peptide fragments generated for determination of the gelonin amino acid sequence. These 19 amino acids may represent a peptide segment that is cleaved from the mature toxin post-translationally, *i.e.* that is not present in the native protein. A similar C-terminal amino acid segment was identified in the plant toxin α-trichosanthin [Chow et al., *J. Biol. Chem., 265*:8670-8674 (1990)]. Therefore, the expression product without the C-terminal fragment is of interest.

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For construction of a gelonin expression vector without the 19 C-terminal amino acids of gelonin, PCR was used to amplify and alter the 3'-end of the gene. pING3728 was amplified with primers gelo-14 and gelo-9 (SEQ ID NO:

5   20). The sequence of primer gelo-14 is set out below.

Gelo-14 (SEQ ID NO: 24)

5' TGATCTCGAGTA<u>CTA</u>TTTAGGATCTTTATCGACGA 3'

Primer gelo-14, which corresponds to gelonin amino acids 245-256, introduces a termination codon (underlined in the

10   primer sequence) in the gelonin gene sequence which stops transcription of the gene before the sequences encoding the terminal 19 amino acids of the gelonin and also introduces a *XhoI* site immediately downstream of the termination codon. The PCR product was cut with *XhoI* and *EcoRI*, and

15   the resulting 208 bp fragment encoding amino acids 185-251 of gelonin was purified from an agarose gel. This fragment was ligated adjacent to the *NcoI* to *EcoRI* fragment from pING3823 encoding amino acids 37-185 of gelonin to generate plasmid pING3732. A final expression vector, pING3734,

20   containing a gelonin gene with an altered 3'-end was generated by substituting an *NcoI* to *XhoI* fragment encoding amino acids 37-251 of gelonin from pING3732 into pING3733.

B.   Identification Of The Native Gelonin 5'-End

Inverse PCR was used to identify a cDNA clone

25   encoding the 5'-end of the mature gelonin gene. 5 μg of total *G. multiflorum* RNA was converted to cDNA using the Superscript Plasmid System (BRL, Gaithersburg, Maryland) with Gelo-11 (SEQ ID NO: 18) as a primer. Gelonin cDNA was self-ligated to generate covalent circular DNA and the

30   ligated DNA was amplified by PCR with oligonucleotides Gelo-9 (SEQ ID NO: 20) and Gelo-16. The sequence of primer Gelo-16 is set out below.

Gelo-16 (SEQ ID NO: 25)

5' GTAAGCAGCATCTGGAGCATCT 3'

35   The PCR product was size-fractionated on an agarose gel and DNAs larger than 300 bp were cloned into *SmaI* cut pUC18.

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Several clones were sequenced with the primer Gelo-18, the sequence of which is set out below.

Gelo-18 (SEQ ID NO: 26)

5' CATTCAAGAAATTCACGTAGG 3'

5   A clone identified as having the largest gelonin-specific insert was designated pING3826. The DNA sequence of pING3826 included the first 32 nucleotides of the natural, mature gelonin gene not necessarily present in gelonin expression plasmids pING3733 and pING3734. The complete

10   DNA sequence of the natural gelonin gene is set out in SEQ ID NO: 11.

C.   Construction Of Expression Vectors
      Containing A Gelonin Gene With A Natural 5' End

      Derivatives of expression vectors pING3733 and

15   pING3734 (described above) containing a gelonin gene with the natural 5' sequence were generated as follows. The 5'-end of gelonin was amplified from pING3826 with the PCR primers Gelo-16 (SEQ ID NO: 24) and Gelo-17, the sequence of which is set out below.

20   Gelo-17 (SEQ ID NO: 27)

              5' GGCCTGGACACCGTGAGCTTTAG 3'

The 285 bp PCR product was treated with T4 polymerase and cut with NcoI. The resulting 100 bp 5'-end DNA fragment was isolated from an agarose gel and ligated adjacent to

25   the 120 bp pelB leader fragment from plC100 (cut with SstI, treated with T4 polymerase and cut with PstI) into either pING3733 or pING3734 digested with PstI and NcoI. The resulting plasmids pING3824 and pING3825 contain the entire native gelonin gene and the native gelonin gene minus the

30   nineteen amino acid carboxyl extension, respectively, linked to the pelB leader and under the transcriptional control of the araB promoter. The gene construct without the nineteen amino acid carboxyl extension in both pING3734 and pING3825 encodes a protein product referred to in this

35   application as "recombinant gelonin".

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D. Purification Of Recombinant Gelonin

Recombinant gelonin was purified by the following procedure: E. coli fermentation broth was concentrated and buffer-exchanged to 10 mM sodium phosphate at pH 7.0 by using an S10Y10 cartridge over a DC10 unit (Amicon) the concentrated and buffer-exchanged material was applied to a CM52 column (100 g, 5X10 cm). The column was washed with 1 L of starting buffer and eluted with a 0 to 300 mM NaCl gradient in starting buffer (750 ml total volume). The pure gelonin containing fractions were pooled (elution was from 100-250 mM NaCl), concentrated over an Amicon YM10 membrane, equilibrated with 10 mM sodium phosphate buffer, pH 7.0, and stored frozen at -20°C. A further purification step was attempted using Blue Toyopearl chromatography. However, this procedure did not result in an increased purity of material and resulted in an approximate 50% loss of the starting material.

Example 3

Assembly Of Gelonin Genes With
Cysteine Residues Available For Conjugation

The wild-type gelonin protein has two cysteine residues at positions 44 and 50 which are linked by an endogenous disulfide bond. The protein contains no free cysteine residue directly available for conjugation to antibodies or other proteins. Analogs of gelonin which contain a free cysteine residue available for conjugation were generated by three different approaches. In one approach, various residues along the primary sequence of the gelonin were replaced with a cysteine residue, creating a series of analogs which contain an odd number of cysteine residues. In another approach, one of the two endogenous cysteines was replaced by alanine, creating a molecule which lacks an intrachain disulfide bond but contains a single, unpaired cysteine. In yet another approach both endogenous cysteines were replaced by alanines and a third non-cysteine residue was replaced by a cysteine, creating an analog with a single, unpaired cysteine.

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Fifteen analogs of gelonin were constructed. Ten non-cysteine residues of gelonin were targeted for substitution with a cysteine residue. Comparison of the amino acid sequence of gelonin to the natural amino acid sequence and tertiary structure of the ricin A-chain (see FIG. 1) suggested that these positions would be at the surface of the molecule and available for conjugation. Each of the ten gelonin analogs include a cysteine substituted in place of one of the following residues: lysine$_{10}$, asparagine$_{60}$, isoleucine$_{103}$, aspartic acid$_{146}$, arginine$_{184}$, serine$_{215}$, asparagine$_{239}$, lysine$_{244}$, aspartic acid$_{247}$, and lysine$_{248}$, and the analogs have respectively been designated Gel$_{C10}$, Gel$_{C60}$, Gel$_{C103}$, Gel$_{C146}$, Gel$_{C184}$, Gel$_{C215}$, Gel$_{C239}$, Gel$_{C244}$, Gel$_{C247}$, and Gel$_{C248}$.

Two analogs of gelonin were constructed in which one of the native gelonin cysteines that participates in an endogenous disulfide bond was replaced with a non-cysteine residue. Specifically, the cysteine at position 50 was replaced with an alanine residue, creating a gelonin analog (designated Gel$_{A50(C44)}$, shown in SEQ ID NO: 99) which has a cysteine available for disulfide bonding at position 44. The Gel$_{A50(C44)}$ analog has been referred to previously as Gel$_{C44}$ (see, e.g., co-owned, co-pending U.S. Patent Application Serial No. 07/988,430, incorporated by reference herein). Conversely, the cysteine at position 44 was replaced with an alanine residue, resulting in an analog (designated Gel$_{A44(C50)}$, shown in SEQ ID NO: 100) which has a cysteine available for disulfide bonding at position 50. The Gel$_{A44(C50)}$ analog has been referred to previously as Gel$_{C50}$ (see, e.g., co-owned, co-pending U.S. Patent Application Serial No. 07/988,430, incorporated by reference herein). The combined series of the foregoing twelve analogs thus spans the entire length of the mature gelonin protein.

Another gelonin analog (Gel$_{A44A50}$ SEQ ID NO: 101) was constructed in which both native gelonin cysteines were replaced with alanines. The Gel$_{A44A50}$ analog has been referred to previously as Gel$_{C44AC50A}$ (see, e.g., co-owned, co-

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pending U.S. Patent Application Serial No. 07/988,430, incorporated by reference herein). Two additional analogs were constructed which have alanine residues substituted in place of both native cysteines and have either a cysteine residue substituted in place of the native lysine at position 10 (Gel$_{C10A44A50}$, shown in SEQ ID NO: 110) or a cysteine residue substituted in place of the native aspartate at position 247 (Gel$_{C247A44A50}$, shown in SEQ ID NO: 111).

The variants of recombinant gelonin were constructed by restriction fragment manipulation or by overlap extension PCR with synthetic oligonucleotides. The sequences of the primers used for PCR are set out below. In each mutagenic primer sequence, the nucleotides corresponding to the changed amino acid, either a cysteine or an alanine residue, are underlined.

Gelo-9   (SEQ ID NO: 20)
Gelo-11   (SEQ ID NO: 18)
Gelo-16   (SEQ ID NO: 25)
Gelo-17   (SEQ ID NO: 27)
Gelo-18   (SEQ ID NO: 26)

Gelo-19   (SEQ ID NO: 58)
5' CAGCCATGGAATCCCATTGCTG 3'

GeloC-1   (SEQ ID NO: 28)
5' TCGATTGCGATCCTAAATAGTACTC 3'

GeloC-2   (SEQ ID NO: 29)
5' TTTAGGATCGCAATCGACGAACTTCAAG 3'

GeloC-3-2 (SEQ ID NO: 30)
5' GTTCGTCTGTAAAGATCCTAAATAGTACTCGA 3'

GeloC-4   (SEQ ID NO: 31)
5' GGATCTTTACAGACGAACTTCAAGAGT 3'

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GeloC-5   (SEQ ID NO: 32)
5' TCTTGTGCTTCGTCGATAAAGATCC 3'

GeloC-6   (SEQ ID NO: 33)
5' ATCGACGAAGCACAAGAGTGCTATTTT 3'

GeloC-9   (SEQ ID NO: 34)
5' GTAAAACCATGCATAGCACTCTTGAAGTTCGT 3'

GeloC-10   (SEQ ID NO: 35)
5' AGTGCTATGCATGGTTTTACTTGATCAACTGC 3'

GeloC-13   (SEQ ID NO: 36)
5' AGCACATGTGGTGCCACTTATATTACCTA 3'

GeloC-14   (SEQ ID NO: 37)
5' TAAGTGGCACCACATGTGCTAAAGCTCACGGTG 3'

GeloC-15   (SEQ ID NO: 38)
5' TGACTGTGGACAGTTGGCGGAAATA 3'

GeloC-16   (SEQ ID NO: 39)
5' GCCAACTGTCCACAGTCATTTGAAAGCGCTACC 3'

GeloC-17   (SEQ ID NO: 40)
5' GATGATCCTGGAAAGGCTTTCGTTTTGGTAGCGCTT3'

GeloC-18   (SEQ ID NO: 41)
5' AAGCCTTTCCAGGATCATCAGC
TTTTTTGCGCAGCAATGGG 3'

GeloC-19   (SEQ ID NO: 42)
5' AAGCCTTTCCAGGATCATCACAT 3'

GeloC-20   (SEQ ID NO: 59)
5' CACATGTAAAACAAGACTTCATTTTGGC 3'

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GeloC-21   (SEQ ID NO: 60)

5' TGAAGTCTTGTTTT<u>AGA</u>TGTGTTTTTGAAGAGGCCT3'

GeloC-22   (SEQ ID NO: 61)

5' ATGCCATA<u>TGC</u>AATTATAAACCAACGGAGA 3'

GeloC-23 (SEQ ID NO: 62)

5' GGTTTATAATT<u>GCA</u>TATGG

CATTTTCATCAAGTTTCTTG 3'

GeloC-24 (SEQ ID NO: 63)

5' CTTTCAACAA<u>TGC</u>ATTCGCCGGCGAATAATAC 3'

GeloC-25   (SEQ ID NO: 64)

5' GCGAAT<u>GCA</u>TTGTTGAAAGTTATTTCTAATTTG 3'

GeloC-26   (SEQ ID NO: 65)

5' GTTT<u>TGT</u>GAGGCAGTTGAATTGGAAC 3'

GeloC-27   (SEQ ID NO: 66)

5' TTCAACTGCCTC<u>ACA</u>AAACATTCCATTTGCACCT 3'

GeloC-28   (SEQ ID NO: 67)

5' AAAA<u>GCT</u>GATGATCCTGGAAAGTG 3'

GeloC-29   (SEQ ID NO: 68)

5' TCCAGGATCATC<u>AGC</u>TTTTTTGCGCAGCAATGGGA 3'

araB2   (SEQ ID NO: 43)

5' GCGACTCTCTACTGTTTC 3'

HINDIII-2   (SEQ ID NO: 44)

5' CGTTAGCAATTTAACTGTGAT 3'

(1) Specifically, a cysteine was introduced at amino acid 247 of gelonin (which is normally occupied by an aspartic acid which corresponds to the cysteine at position

259 in the ricin A-chain) by PCR with mutagenic primers GeloC-3-2 and GeloC-4 in conjunction with primers *HINDIII-2* (a primer located in the vector portion of pING3734 or pING3825), Gelo-9 and Gelo-8. Template DNA (pING3734) was

5   amplified with GeloC-3-2 and *HINDIII-2* and in a concurrent reaction with GeloC-4 and Gelo-9. The products of these reactions were mixed and amplified with the outside primers Gelo-8 and *HINDIII-2*. The reaction product was cut with *EcoRI* and *XhoI*, purified, and was inserted into plasmid

10   pING3825 in a three-piece ligation. The DNA sequence of the Gel_{C247} variant (SEQ ID NO: 102) was then verified. The plasmid containing the sequence encoding Gel_{C247} was designated pING3737 and was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville,

15   MD 20852 on June 9, 1992 as ATCC Accession No. 69009.

(2-3) In the same manner, a cysteine residue was introduced in place of the amino acid at position 248 (a lysine) of gelonin with the mutagenic oligonucleotides GeloC-1 and GeloC-2 to generate analog Gel_{C248} (SEQ ID NO:

20   103) in plasmid pING3741, and a cysteine residue was introduced at amino acid position 239 (normally occupied by a lysine) with primers GeloC-9 and GeloC-10 to generate analog Gel_{239} (SEQ ID NO: 104) in plasmid pING3744.

(4) Also in the same manner, a cysteine residue

25   was introduced at amino acid 244 (a lysine) of gelonin with mutagenic primers GeloC-5 and GeloC-6 to generate analog Gel_{C244} (SEQ ID NO: 105) in a plasmid designated pING3736. This variant was prepared by PCR using plasmid pING3734 as template DNA rather than pING3825. It therefore encodes

30   the same N-terminal gelonin amino acid sequence as plasmids pING3737, pING3741, and pING3744, but includes the PCR primer-derived 5'-end 32 nucleotides instead of the native gelonin 5'-end nucleotides.

(5) A cysteine residue was introduced in place of

35   the amino acid (normally occupied by a lysine) at position 10 of gelonin by a similar procedure. A cysteine was introduced with mutagenic primers GeloC-13 and GeloC-14 by

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amplifying pING3824 with araB2 (a vector primer) and GeloC-14, and in a separate reaction, with GeloC-13 and Gelo-11. ~~These reaction products were mixed and amplified~~ with the outside primers araB2 and Gelo-11. The PCR product was cut

5   with *Pst*I and *Nco*I, purified, and cloned back into pING3825 to generate analog Gel_{C10} (SEQ ID NO: 106) in the plasmid designated pING3746 and deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 on June 9, 1992 as ATCC Accession No. 69008.

10   (6) The asparagine at position 60 of gelonin was replaced with a cysteine residue using two mutagenic oligos, GeloC-15 and GeloC-16, in conjunction with oligos araB2 and Gelo-11 in the same manner as for the Gel_{C10} variant. The plasmid encoding the Gel_{C60} (SEQ ID NO: 107)

15   analog was designated pING3749.

             (7) A cysteine was introduced at amino acid 103 (an isoleucine) by PCR with mutagenic primers GeloC-20 and GeloC-21 in conjunction with primers araB2 and *HIND*III-2. Template DNA (pING3733) was amplified with GeloC-21 and

20   araB2 and separately with GeloC-20 and *HIND*III-2.   The products of these reactions were mixed and amplified with the outside primers araB2 and *HIND*III-2.   The reaction product was cut with *Nco*I and *Bcl*I, purified, and inserted into pING3825 digested with *Nco*I and *Bcl*I.   The

25   oligonucleotides used to place a cysteine at residue 103 also introduced an *Afl*III restriction site which was verified in the cloned gene. The plasmid containing the Gel_{C103} (SEQ ID NO: 108) analog was designated pING3760.

             (8) A cysteine was introduced at position 146

30   (an aspartic acid) by a similar strategy. Template DNA (pING3733) was amplified with mutagenic primer GeloC-22 and Gelo-14 and separately with mutagenic primer GeloC-23 and Gelo-19. The products of these reactions were mixed, and amplified with Gelo-19 and Gelo-14. The reaction product

35   was cut with *Bgl*II and *Eco*RI, and can be inserted into pING3825 in a three-piece ligation. The oligonucleotides used to place a cysteine at residue 146 also introduced a

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*Nde*I restriction site which can be verified in the cloned gene.

(9)   To introduce a cysteine at position 184 (normally occupied by an arginine) of gelonin, template DNA (pING3733) was amplified with mutagenic primer GeloC-25 and *ara*B-2 and separately with mutagenic primer GeloC-24 and *HIND*III-2. The products of these reactions were mixed, and amplified with *ara*B2 and Gelo-14. The reaction product was cut with *Nco*I and *Bcl*I, and inserted into pING3825 previously digested with *Nco*I and *Bcl*I.   The oligonucleotides used to place a cysteine at residue 184 also introduced an *Nsi*I restriction site which was verified in the cloned gene. The plasmid containing the sequence encoding the Gel_{C184} (SEQ ID NO: 109) variant was designated pING3761.

(10) A cysteine may be introduced at position 215 (a serine) by a similar strategy. Template DNA (pING3733) was amplified with mutagenic primer GeloC-27 and *ara*B2 and separately with mutagenic primer GeloC-26 and *HIND*III-2. The products of these reactions were mixed, and amplified with *ara*B2 and *HIND*III-2. The reaction product was cut with *Eco*RI and *Bcl*I, and may be inserted into pING3825 in a three-piece ligation.

(11) Another gelonin variant with a free cysteine residue was generated by replacing one of the two naturally occurring gelonin cysteine residues, the cysteine a position 50, with an alanine. Plasmid pING3824 was amplified with primers GeloC-17 and Gelo-11, and concurrently in a separate reaction with primers GeloC-19 and *ara*B2. The reaction products were mixed and amplified with *ara*B2 and Gelo-11. This product was cut with *Nco*I and *Bgl*II, and cloned back into the vector portion of pING3825 to generate pING3747 (ATCC 69101).   This analog was designated $Gel_{A50(C44)}$ and it contains a cysteine available for disulfide bonding at amino acid position 44. Non-cysteine residues, other than alanine, which do not disrupt the activity of gelonin, also may be inserted at position 50 in

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natural gelonin in order to generate a gelonin analog with a single cysteine at position 44.

(12)   A gelonin variant in which the natural cysteine at position 44 was changed to alanine was constructed by amplifying pING3733 using the mutagenic oligonucleotides GeloC-28 and GeloC-29 in conjunction with primers araB2 and HINDIII-2.   The amplified DNA was cut with NcoI and BglII and cloned into a gelonin vector, generating pING3756. That variant generated was designated Gel$_{A44(C50)}$. Non-cysteine residues, other than alanine, which do not disrupt gelonin activity, also may be inserted at position 44 in order to generate a gelonin analog with a single cysteine at position 50.

(13) · A gelonin variant in which both the cysteine at position 44 and the cysteine at position 50 of gelonin were changed to alanine residues was constructed by overlap   PCR   of   pING3824   using   the   mutagenic oligonucleotides GeloC-17 and GeloC-18 in conjunction with primers araB2 and Gelo-11.   This analog, like the native gelonin protein, has no cysteine residues available for conjugation.   The   plasmid   encoding   the   analog   was designated pING3750.   The analog generated was designated Gel$_{A44A50}$ (SEQ ID NO: 101). Non-cysteine residues, other than alanine, which do not disrupt gelonin activity, also may be substituted   at   both   positions   44   and   50   in   order   to generate a gelonin analog with no cysteine residues.

(14)   The triple mutant Gelonin$_{C247A44A50}$ (SEQ ID NO: 111) was constructed from the plasmids pING3824, pING3750 and pING3737. This variant contains an introduced cysteine at   position   247   while   both   of   the   naturally   occurring cysteine residues at positions 44 and 50 have been replaced with alanine.   The analog is desirable because, in this analog, disulfide linkage to an antibody is only assured at a single cysteine residue.   Plasmid pING3824 was cut with NcoI and XhoI and the vector fragment was purified in an agarose gel.   pING3750 was cut with NcoI and EcoRI and pING3737 was cut with EcoRI and XhoI.   The NcoI-EcoRI

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fragment encodes the alanines at positions 44 and 50 while the *EcoRI-XhoI* fragment encodes the cysteine at position 247. Each of these fragments was purified and ligated to the *NcoI* to *XhoI* vector fragment. The resulting plasmid is
5   named pING3752.

(15) The triple mutant Gelonin$_{C10A44A50}$ (SEQ ID NO: 110) was also constructed by assembly from previously assembled plasmids. In this case, pING3746 was cut with *PstI* and *NcoI*, while pING3750 was cut with *NcoI* and *XhoI*.
10   Each of the insert fragments were purified by electrophoresis in an agarose gel, and the fragments were ligated into a *PstI* and *XhoI* digested vector fragment. The resulting vector was designated pING3753. The Gel$_{C10A44A50}$ analog has been referred to previously as Gel$_{C10C44AC50A}$ (*see,*
15   *e.g.*, co-owned, co-pending U.S. Patent Application Serial No. 07/988,430, incorporated by reference herein).

Each of the gelonin variants constructed was transformed into *E. coli* strain E104. Upon induction of bacterial cultures with arabinose, gelonin polypeptide
20   could be detected in the culture supernatants with gelonin-specific antibodies. There were no significant differences detected in the expression levels of gelonin from plasmids pING3734 and pING3825, or in the levels from any of the gelonin variants. Each protein was produced in *E. coli* at
25   levels of approximately 1 g/l.

Example 4

Reticulocyte Lysate Assay

The ability of gelonin and recombinant gelonin analogs to inhibit protein synthesis *in vitro* was tested
30   using a reticulocyte lysate assay (RLA) described in Press *et al.*, *Immunol. Letters*, *14*:37-41 (1986). The assay measures the inhibition of protein synthesis in a cell-free system using endogenous globin mRNA from a rabbit red blood cell lysate. Decreased incorporation of tritiated leucine
35   (^{3}H-Leu) was measured as a function of toxin concentration. Serial log dilutions of standard toxin (the 30 kD form of

ricin A-chain, abbreviated as RTA 30), native gelonin, recombinant gelonin (rGelonin or rGel) and gelonin analogs were tested over a range of 1 μg/ml to 1 pg/ml. Samples were tested in triplicate, prepared on ice, incubated for

5   30 minutes at 37°C, and then counted on an Inotec Trace 96 cascade ionization counter. By comparison with an uninhibited sample, the picomolar concentration of toxin (pM) which corresponds to 50% inhibition of protein synthesis (IC_{50}) was calculated. As is shown in Table 1

10   below, recombinant gelonin and most of its analogs exhibit activity in the RLA comparable to that of native gelonin. For some of the analogs (such as Gel_{c239}), RLA activity was diminished.

Table 1

15 | Toxin | | $IC_{50}(pM)$ |
|---|---|---|
| RTA 30 | | 2.5 |
| Gelonin | | 15 |
| rGelonin | | 11 |
| Gel_{C10} | | 60 |
20 | $Gel_{A50(C44)}$ | 20 | |
| $Gel_{A44(C50)}$ | 47 | |
| Gel_{C60} | | 26 |
| Gel_{C239} | | 955 |
| Gel_{C244} | | 32 |
25 | Gel_{C247} | | 12 |
| Gel_{C248} | | 47 |
| Gel_{A44A50} | | 16 |
| $Gel_{C10A44S0A}$ | 7 | |
| $Gel_{C247A44A50}$ | 20 | |

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Example 5

Human-Engineered Antibodies
For Construction Of Immunotoxins

Antibodies for use in constructing immunotoxins
according to the present invention may be humanized
antibodies, such as he3 and fragments thereof which display
increased content of human amino acids and a high affinity
for human CD5 cell differentiation marker. he3 is a
humanized form of a mouse H65 antibody (H65 is a preferred
monoclonal antibody for use in preparing humanized
antibodies according to the present invention and is
produced by hybridoma cell line XMMLY-H65 (H65) deposited
with the American Type Culture Collection in Rockville,
Maryland (A.T.C.C.) and given the Accession No. HB9286).

Humanized antibodies for use in the present
invention are prepared as disclosed herein using the
humanized forms of the murine H65 antibody in which both
low and moderate risk changes described below were made in
both variable regions. Such humanized antibodies should
have less immunogenicity and have therapeutic utility in
the treatment of autoimmune diseases in humans. For
example, because of their increased affinity over existing
therapeutic monoclonal antibodies such as H65, he3
antibodies of the invention may be administered in lower
doses than H65 anti-CD5 antibodies in order to obtain the
same therapeutic effect.

Humanized antibodies, such as he3, are useful in
reducing the immunogenicity of foreign antibodies and also
results in increased potency when used as a portion of an
immunoconjugate.

Construction of humanized antibody variable
domains according to the present invention and for use as
components of immunotoxins may be based on a method which
includes the steps of: (1) identification of the amino acid
residues of an antibody variable domain which may be
modified without diminishing the native affinity of the
domain for antigen while reducing its immunogenicity with
respect to a heterologous species; and (2) the preparation

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of antibody variable domains having modifications at the identified residues which are useful for administration to heterologous species. The methods of the invention are based on a model of the antibody variable domain described herein which predicts the involvement of each amino acid in the structure of the domain.

Unlike   other   methods   for   humanization   of antibodies, which advocate replacement of the entire classical antibody framework regions with those from a human antibody, the methods described herein introduce human residues into the variable domain of an antibody only in positions which are not critical for antigen-binding activity   and   which   are   likely   to be   exposed   to immunogenicity-stimulating factors. The present methods are designed to retain sufficient natural internal structure of the variable domain so that the antigen-binding capacity of the modified domain is not diminished in comparison to the natural domain.

The human consensus sequences in which moderate risk residues are converted from mouse residues to human residues are represented in Figures 10A and 10B as lines labelled hK1 (*i.e.*, subgroup 1 of the human kappa chain) and hH3 (*i.e.*, subgroup 3 of the human heavy chain). Symbols in the figures for conservation and for risk in "bind" and "bury" lines are follows:

> First Symbol in Pair (Ligand Binding)
> +   Little or not direct influence on antigen-binding loops, low risk if substituted
> °   Indirectly involved in antigen-binding loop structure, moderate risk if changed
> −   Directly involved in antigen-binding loop conformation or antigen contact, great risk if modified

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Second Symbol in Pair (Immunogenicity/Struture)

+   Highly accessible to solvent, high immunogenicity, low risk if substituted

°   Partially buried, moderate immunogenicity, moderate risk if altered

-     Completely buried in subunit's hydrophobic core, low immunogenicity, high risk if changed

=   Completely buried in interface between subunits, low immunogenicity, high risk if modified

Significance of Pairs

++   Low risk

Highly accessible to solvent and high immunogenicity, but little or no effect on specific antigen binding

°+, +°, °° Moderate Risk

Slight immunogenicity or indirect involvment with antigen binding

any - or = High risk

Buried within the subunit core/ interface or strongly involved in antigen binding, but little immunogenic potential

In the line labelled "mod", a dot (.) represents a residue which may be mutated from "mouse" to "human" at moderate risk. There are 29 such moderate risk positions.

The mouse residue matches the human consensus residue more than 50% of the time at 131 positions (102 positions match 90%-100% and 29 positions match 50% to 90%). These positions were not changed.

The lines labelled M/H in Figures 12A and 12B indicate the 91 positions which differed significantly between the mouse and human sequences (i.e., where the

human sequences have the mouse residue less than 50% of the time). Moderate risk positions, designated m in the M/H line, were kept "mouse"; whereas those designated H or h were changed to human. The 25 low risk positions which were already human-like or which were previously humanized (as described *supra* in Example 2) are designated " ^ " in the M/H line. Finally, the 54 high risk positions in which the mouse and human residues did not match are designated M and are kept "mouse".

Fifteen differences occur at moderate risk positions at which the mouse and human sequences differ. At ten of those positions (designated "H" on the M/H lines of Figure 6) the mouse residue aligns with a human consensus amino acid which is highly conserved. Therefore, the mouse residue at that position is identified as one to be changed to the conserved human·residue.

At moderate risk positions (designated "m") in which the mouse and the human sequences differ, the mouse residue aligns with a human consensus amino acid which is moderately conserved. However, since the mouse residue is found at that position in other actual sequences of human antibodies [*See* Kabat, *et al.*, sequences of Proteins of Immunoglobulin Interest,Fourth Edition, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health (1987)] the positions are identified as ones to be kept "mouse." Although there are no such positions in this particular sequence, such positions may occur in other antibodies.

At four moderate risk positions (designated "h"), the mouse residue aligns with a human consensus amino acid which is moderately conserved but the mouse residue is not found at that position in an actual human antibody sequence in Kabat, *et al. Sequences of Proteins of Immunoglobulin Interest, supra.* Therefore, that position is identified as ones to be changed to "human."

At one moderate risk position (designated "m") in which the mouse and human sequences differ, the mouse

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residue aligns with a human consensus amino acid which is poorly conserved. Therefore, that position is identified as one to be kept "mouse."

A.   Assembly Of Moderate Risk
     Heavy Chain Expression Vectors

The humanized H65 heavy chain containing the moderate risk residues was assembled by the following strategy. The moderate-risk expression vector was assembled from intermediate vectors. The six oligonucleotide sequences (oligos), disclosed in Figure 12 and labelled HUH-G11, HUH-G12, HUH-G3, HUH-G4, HUH-G5, and HUH-G6 (the sequences of HUH-G11 and HUH-G12 are set out in SEQ ID Nos. 131 and 132 and HUH-G3, HUH-G4, HUH-G5, and HUH-G6 are set out in SEQ ID NOS: 137-140) were assembled by PCR. Oligonucleotides containing the synthetic humanized antibody gene were mixed in pairs (HUH-G11 + HUH-G12, HUH-G3 + HUH-G4, and HUH-G5 + HUH-G6) in a 100 μl reaction with 1 μg of each DNA and filled in as described above. A portion of each reaction product was mixed in pairs (HUH-G11, 12 + HUH-G3, 4; HUH-G3, 4 + HUH-G5, 6), 2.5 U Taq was added and samples were reincubated as described above. The V-J region was assembled by mixing equal amounts of the HUH-G11, 12, 3, 4 reaction product with the HUH-G3, 4, 5, 6 product, followed by PCR with 0.5 ug of primers H65G-2S and H65-G2 as described above. The reaction product was cut with *SalI* and *BstEII* and cloned into the expression vector, similar to that described for heavy chain in Robinson *et al.*, *Hum. Antibod. Hybridomas* 2:84 (1991), generating pING4617. That plasmid was sequenced with Sequenase (USB, Cleveland), revealing that two residues were altered (a G-A at position 288 and a A-T at position 312, numbered from the beginning of the leader sequence). The correct variable region was restored by substitution of this region from pING4612, generating the expected V-region sequence in pING4619.

An intermediate vector containing the other moderate-risk changes was constructed by PCR assembly of

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the oligos HUH-G13, HUH-G14, HUH-G15, and HUH-G16 (Fig. 11 and SEQ ID Nos: 133-136). Oligos HUH-G13 + HUH-G14 and HUH-G15 + HUH-G16 were mixed and filled in with Vent polymerase (New England Biotabs) in a reaction containing

5   10 mM KCl, 20 mM TRIS pH 8.8, 10 mM $(NH_4)_2SO_2$, 2mM $MgSO_4$, 0.1% Triton X-100, 100 ng/ml BSA, 200 uM of each dNTP, and 2 units of Vent polymerase in a total volume of 100 μl. The reaction mix was incubated at 94°C for 1 minute, followed by 2 minutes at 50°C and 20 minutes at 72°C. The

10   reaction products (40 μl) were mixed and amplified with the oligonucleotides H65-G13 and H65-G2 with Vent polymerase in the same reaction buffer and amplified for 25 cycles with denaturation at 94°C for 1 minute, annealing at 50°C for 2 minutes and polymerization at 72°C for 3 minutes. The

15   reaction product was treated with T4 polymerase and then digested with AccI. The 274 base pair (bp) fragment was purified on an agarose gel and ligated along with the 141 bp SalI to AccI fragment from pING4619 into pUC18 cut with SalI and SmaI to generate pING4620. pING4620 contains the

20   entire signal sequence, V-region, and J-region of the moderate-risk H65 heavy chain.

The final expression vector for the moderate-risk H65 heavy chain, pING4621, was assembled by cloning the SalI to BstEII fragment from pING4620 into the same

25   expression vector described above.

B.   Assembly Of Moderate-Risk
      Light Chain Expression Vectors

The moderate-risk humanized V- and J-segments of the light chain were assembled from six oligonucleotides,

30     $H65K-1 (SEQ ID NO: 117), HUH-K7 (SEQ ID NO: 119), HUH-K6 (SEQ ID NO: 118), HUH-K8 (SEQ ID NO: 120), HUH-K4 (SEQ ID NO: 121 and HUH-K5 (SEQ ID NO: 122). The oligonucleotides were amplified with PCR primers H65K-2S and JK1-HindIII. Oligonucleotides containing the synthetic humanized

35   antibody gene were mixed in pairs ($H65-K1 + HUH-K7, HUH-K6 + HUH-K4 + HUH-K5) and incubated with Vent polymerase as described for the moderate-risk heavy chain. A portion of

each reaction product (40 ul) was mixed in pairs ($H65H-K1/HUH-K7 + HUH-K6, 8; HUH-K6, 8 + HUH-K4, 5) and filled in as above. The light chain gene was then assembled by amplifying the full length gene with the PCR primers H65K-2S and JK1-*Hind*III with Vent polymerase for 25 cycles as outlined above. The assembled V/J region was cut with *Sal*I and *Hind*III, purified by electrophoresis on an agarose gel, and assembled into a light chain antibody expression vector, pING4630.

Example 6

Transfection Of he3 Genes And
Purification Of Expression Products

A.   Stable Transfection Of Mouse Lymphoid
      Cells For The Production Of he3 Antibody

The cell line Sp2/0 (American Type Culture Collection Accession No. CRL1581) was grown in Dulbecco's Modified Eagle Medium plus 4.5 g/l glucose (DMEM, Gibco) plus 10% fetal bovine serum. Media were supplemented with glutamine/penicillin/streptomycin (Irvine Scientific, Irvine, California).

The electroporation method of Potter, H., *et al.*, *Proc. Natl. Acad. Sci., USA*, 81:7161 (1984) was used. After transfection, cells were allowed to recover in complete DMEM for 24-48 hours, and then seeded at 10,000 to 50,000 cells per well in 96-well culture plates in the presence of selective medium. Histidinol (Sigma) selection was at 1.71 μg/ml, and mycophenolic acid (Calbiochem) was at 6 μg/ml plus 0.25 mg/ml xanthine (Sigma). The electroporation technique gave a transfection frequency of 1-10 x 10^{-5} for the Sp2/0 cells.

The he3 light chain expression plasmid pING4630 was linearized by digestion with *Pvu*I restriction endonuclease and transfected into Sp2/0 cells, giving mycophenolic acid - resistant clones which were screened for light chain synthesis.

Four of the top-producing subclones, secreting 4.9-7.5 μg/ml were combined into two pools (2 clones/pool)

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and each pool was transfected with plasmid pING42621, containing the moderate-risk heavy chain. After selection with histidinol, the clones producing the most light plus heavy chain, Sp2/0-4630 and 4621 Clones C1705 and C1718,
5   secreted antibody at approximately 15 and 22 μg/ul respectively in the presence of 10^{-7} M dexamethasone in an overgrown culture in a T25 flask. Clone C1718 was deposited with the American Type Culture Collection, 1230 Parklawn Drive, Rockville, Maryland, 20852 on December 1,
10   1992 as ATCC HB 11206. The best producer is a subclone of Clone C1718 which is produced by limiting dilution subcloning of Clone C1718.

B.   Purification Of he3 Antibody
     Secreted In Tissue Culture

15   Sp2/0-4630 + 4621 Clone C1705cells were grown in culture medium HB101 (Hana Biologics)+ 1% Fetal Bovine Serum, supplemented with 10 mM HEPES, 1x Glutamine-Pen-Strep (Irvine Scientific #9316). The spent medium was centrifuged at about 5,000 x g for 20 minutes. The
20   antibody level was measured by ELISA. Approximately 200 ml of cell culture supernatant was loaded onto a 2 ml Protein A-column (Sigma Chemicals), equilibrated with PBS (buffer 0.15 M NaCl, 5 mM sodium phosphate, 1 mM potassium phosphate, buffer pH 7.2). The he3 antibody was eluted
25   with a step pH gradient (pH 5.5, 4.5 and 2.5). A fraction containing he3 antibody (9% yield) but not bovine antibody, was neutralized with 1 M Tris pH 8.5, and then concentrated 10-fold by Centricon 30 (Amicon) diluted 10-fold with PBS, reconcentrated 10-fold by Centricon 30, diluted 10-fold
30   with PBS, and finally reconcentrated 10-fold. The antibody was stored in 0.25 ml aliquots at -20° C.

C.   Affinity Measurements Of he3 IgG For CD5
          The affinity of he3 IgG for CD5 was determined using Molt-4M cells, which express CD5 on their surface,
35   and I^{125}-labeled chimeric H65 IgG in a competitive binding assay. Culture supernatants from Clone C1705 and C1718 and

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purified IgG from C1705 were used as the sources of he3 IgG.

For this assay, 20 μg of chimeric H65 IgG (cH65 IgG) was iodinated by exposure to 100 μl lactoperoxidase-glucose oxidase immobilized beads (Enzymobeads, BioRad), 100 μl of PBS, 1.0 mCi I^{125} (Amersham, IMS30), 50 μl of 55 mM b-D-glucose for 45 minutes at 23°C. The reaction was quenched by the addition of 20 μl of 105 mM sodium metabisulfite and 120 mM potassium iodine ≈ followed by centrifugation for 1 minute to pellet the beads. ^{125}I-cH65 IgG was purified by gel filtration using 7 mls of sephadex G25, using PBS (137 mM NaCl, 1.47 mM KH$_2$PO$_4$, 8.1 mM Na$_2$HPO$_4$, 2.68 mM KCl at pH 7.2-7.4) plus 0.1% BSA. ^{125}I-cH65 IgG recovery and specific activity were determined by TCA precipitation.

Competitive binding was performed as follows: 100 μl of Molt-4M cells were washed two times in ice-cold DHB binding buffer (Dubellco's modified Eagle's medium (Gibco, 320-1965PJ), 1.0% BSA and 10 mM Hepes at pH 7.2. -7.4). Cells were resuspended in the same buffer, plated into 96 v-bottomed wells (Costar) at 3 x 10^5 cells per well and pelleted at 4°C by centrifugation for 5 min at 1,000 rpm using a Beckman JS 4.2 rotor; 50 μl of 2X-concentrated 0.1 nM ^{125}I-cH65 IgG in DHB was then added to each well and competed with 50 μl of 2X - concentrated cH65 IgG or humanized antibody in DHB at final antibody concentrations from 100 nM to 0.0017 nM. Humanized antibody was obtained from culture supernatants of Sp2/0 clone C1718 which expresses he3 IgG. The concentration of the antibody in the supernatants was established by ELISA using a chimeric antibody as a standard. The concentration of the antibody in the purified preparation was determined by binding was allowed to proceed at 4°C for 5 hrs and was terminated by washing cells three times with 200 μl of DHB binding buffer by centrifugation for 5 min at 1,000 rpm. All buffers and operations were at 4°C. Radioactivity was determined by solubilizing cells in 100 μl of 1.0 M NaOH and counting in

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a Cobra II auto gamma counter (Packard). Data from binding experiments were analyzed by the weighted nonlinear least squares curve fitting program, MacLigand, a Macintosh version of the computer program "Ligand" from Munson,

5   *Analyt. Biochem.*, 107:220 (1980). Objective statistical criteria (F, test, extra sum squares principle) were used to evaluate goodness of fit and for discriminating between models. Nonspecific binding was treated as a parameter subject to error and was fitted simultaneously with other

10   parameters.

Figure 11, provides data showing relative binding of he3 and CH65 to CD5 on molt-4M cells in a competition binding assay. These results demonstrate that the moderate-risk changes made in he3 IgG result in an

15   antibody with a higher affinity than the chimeric mouse-human form of this antibody (cH65) for its target, CD5.

Example 7

Preparation of Gelonin Immunoconjugates

Gelonin analogs of the invention were variously

20   conjugated to murine (ATCC HB9286) and chimeric H65 (cH65) antibody, cH65 antibody domains (including cFab, cFab' and $cF(ab')_2$ fragments), and humanized antibodies and antibody domains, all of which are specifically reactive with the human T cell determinant CD5. H65 antibody was prepared

25   and purified by methods described in U.S. Patent Application Serial No. 07/306,433, *supra* and International Publication No. WO 89/06968, *supra*. Chimeric H65 antibody was prepared according to methods similar to those described in Robinson *et al., Human Antibodies and*

30   *Hybridomas,* 2:84-93 (1991), incorporated by reference herein. Chimeric H65 Fab, Fab', and $F(ab')_2$ proteins were prepared as described in Better, *et al., Proc. Nat. Acad. Sci. (USA), 90:* 457-461 (1993), incorporated by reference herein. Finally, he3 humanized antibodies were prepared

35   according to the procedures described in U.S. Patent

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Application Serial No. 07/808,464, incorporated by reference herein.

A.   Conjugation To H65 Antibodies

To expose a reactive sulfhydryl, the unpaired cysteine residues of the gelonin analogs were first reduced by incubation with 0.1 to 2 mM DTT (30-60 minutes at room temperature), and then were desalted by size-exclusion chromatography.

Specifically, the Gel_{C248} analog (3.8 mg/ml) was treated with 2 mM DTT for 60 minutes in 0.1 M Naphosphate, 0.25 M NaCl, pH 7.5 buffer. The Gel_{C244} variant (7.6 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M Naphosphate, 0.25 M NaCl, pH 7.5 buffer. The Gel_{C247} analog (4 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 M Naphosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The Gel_{C239} variant (3.2 mg/ml) was treated with 2 mM DTT for 30 minutes in 0.1 m Naphosphate, 0.5 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. The $Gel_{A50(C44)}$ analog (4.2 mg/ml) was treated with 0.1 mM DTT for 30 minutes in 0.1 M Naphosphate, 0.1 M NaCl, pH 7.5 buffer with 0.5 mM EDTA. Lastly, the Gel_{C10} variant (3.1 mg/ml) was treated with 1 mM DTT for 20 minutes in 0.1 M Naphosphate, 0.1 M NaCl, pH 7.5 buffer with 1 mM EDTA.

The presence of a free sulfhydryl was verified by reaction with DTNB and the average value obtained was 1.4 ± 0.65 SH/molecule. No free thiols were detected in the absence of reduction.

H65 antibody and chimeric H65 antibody were chemically modified with the hindered linker 5-methyl-2-iminothiolane (M2IT) at lysine residues to introduce a reactive sulfhydryl group as described in Goff et al., Bioconjugate Chem., 1:381-386 (1990) and co-owned Carroll et al., U.S. Patent No. 5,093,475, incorporated by reference herein.

Specifically, for conjugation with Gel_{C248} and Gel_{C244}, murine H65 antibody at 4 mg/mL was derivitized with

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18x M2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM NaCl, pH 8 buffer for 1 hour at 23°C. The reaction gave 1.9 linkers per antibody as determined by DTNB assay.

5   For conjugation with Gel_{C247} and Gel_{C239}, H65 antibody at 4.7 mg/mL was derivitized with 20x M2IT and 2.5 mM DTNB in 25 mM TEOA 150 mM NaCl, pH 8 buffer for 50 minutes at 23°C. The reaction gave 1.6 linkers per antibody as determined by DTNB assay.

Before reaction with $Gel_{A50(C44)}$, H65 antibody at 5.8
10   mg/mL was derivitized with 20x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM NaCl, pH 8 buffer for 30 minutes at 23°C. The reaction gave 1.5 linkers per antibody as determined by DTNB assay.

For conjugation with Gel_{C10}, H65 antibody at 2.2
15   mg/mL was derivitized with 10x m2IT and 2.5 mM DTNB in 25 mM TEOA, 150 mM NaCl, pH 8 buffer for 1 hour at 23°C. The reaction gave 1.4 linkers per antibody as determined by DTNB assay.

Chimeric H65 antibody was prepared for
20   conjugation in a similar manner to murine H65 antibody.

Two methods were initially compared for their effectiveness in preparing immunoconjugates with recombinant gelonin. First, the native disulfide bond in recombinant gelonin was reduced by the addition of 2mM DTT
25   at room temperature for 30 minutes. The reduced gelonin was recovered by size-exclusion chromatography on a column of Sephadex GF-05LS and assayed for the presence of free sulfhydryls by the DTNB assay. 1.4 free SH groups were detected. This reduced gelonin was then reacted with H65-
30   (M2IT)-S-S-TNB (1.8 TNB groups/H65). Under these experimental conditions, little or no conjugate was prepared between reduced gelonin and thiol-activated H65 antibody.

In contrast, when both the recombinant gelonin
35   and the H65 antibody were first derivitized with the crosslinker M2IT (creating gelonin-(M2IT)-SH and H65-(M2IT)-S-S-TNB) and then mixed together, H65-(M2IT)-S-S-

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(M2IT)-gelonin conjugate was prepared in good yield (toxin/antibody ratio of 1.6). The starting materials for this conjugation (gelonin-(M2IT)-SH and H65-(M2IT)-S-S-TNB) contained linker/protein ratios of 1.2 and 1.4,
5 respectively. Native gelonin was derivatized in a similar manner prior to conjugation to murine or chimeric H65 antibody.

The reduced gelonin analogs were mixed with H65-(M2IT)-S-S-TNB to allow conjugation. The following
10 conjugation reactions were set up for each analog: 23 mg (in 7.2 ml) of H65-M2IT-TNB were mixed with a 5-fold molar excess of Gel_{C248} (23 mg in 6 ml) for 2 hours at room temperature, then for 18 hours overnight at 4°C; 23 mg (in 7.3 ml) of H65-m2IT-TNB were mixed with a 5-fold molar
15 excess of Gel_{C244} (23 mg in 3 ml) for 3 hours at room temperature, then for 18 hours overnight at 4°C; 9 mg (in 2.8 mL) of H65-m2IT-TNB were mixed with a 5-fold molar excess of Gel_{C247} (9 mg in 2.25 mL) for 2 hours at room temperature, then for 5 nights at 4°C; 9 mg (in 2.8 mL) of
20 H65-m2IT-TNB were mixed with a 5-fold molar excess of Gel_{C239} (9mg in 2.6 mL) for 2 hours at room temperature, then at 4°C for 3 days; 12 mg (in 1.9 mL) of H65-m2IT-TNB were mixed with a 5.6-fold molar excess of $Gel_{A50(C44)}$ (13.44 mg in 3.2 mL) for 4.5 hours at room temperature, then 4°C
25 overnight; and 11 mg of H65-m2IT-TNB were mixed with a 5-fold molar excess of Gel_{C10} (11 mg in 3.5 mL) for 4 hours at room temperature, then at 4°C overnight.

Following conjugation, unreacted M2IT linkers on the antibody were quenched with 1:1 mole cysteamine to
30 linker for 15 minutes at room temperature. The quenched reaction solution was then loaded onto a gel filtration column [Sephadex G-150 (Pharmacia) in the case of Gel_{C248}, Gel_{C247}, Gel_{C244} and Gel_{C239} and an AcA-44 column (IBF Biotecnics, France) in the case of $Gel_{A50(C44)}$ and Gel_{C10}]. The
35 reactions were run over the gel filtration columns and eluted with 10 mM Tris, 0.15M NaCl pH 7. The first peak off each column was loaded onto Blue Toyopearl® resin

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(TosoHaas, Philadelphia, Pennsylvania) in 10 mM Tris, 30 mM NaCl, pH 7 and the product was eluted with 10 mM Tris, 0.5 M NaCl, pH 7.5.

Samples of the final conjugation products were run on 5% non-reduced SDS PAGE, Coomassie stained and scanned with a Shimadzu laser densitometer to quantitate the number of toxins per antibody (T/A ratio). The yield of final product for each analog conjugate was as follows: Gel_{C248}, 17 mg with a T/A ration of 1.6; Gel_{C247}, 1.1 mg with a T/A ratio of 1; Gel_{C244}, 4.5 mgs with a T/A ratio of 1.46; Gel_{C239}, 2.9 mg with a T/A ratio of 2.4; $Gel_{A50(C44)}$, 7.3 mg with a T/A ratio of 1.22; and Gel_{C10}, 6.2 mg with a T/A ratio of 1.37. Conjugation efficiency (*i.e.*, conversion of free antibody to immunoconjugate) was significantly greater (~80%) for some analogs (Gel_{C10}, $Gel_{A50(C44)}$, Gel_{C239}, Gel_{C247}, and Gel_{C248}) than for others (~10%, Gel_{C244}).

B. Gelonin Immunoconjugates With
   Chimeric And Humanized Antibodies

Analogs Gel_{C247} and $Gel_{A50(C44)}$ were also conjugated to various chimeric [cH65Fab, cH65Fab' and cH65F(ab')$_2$] and "human engineered" [he1 Fab, he2-Fab, he3-Fab, he1 Fab' and he1 F(ab')$_2$] antibody fragments. Chimeric H65 antibody fragments may be prepared according to the methods described in International Publication No. WO 89/00999, *supra*. The DNA sequences encoding the variable regions of H65 antibody fragments that were human engineered (referring to the replacement of selected murine-encoded amino acids to make the H65 antibody sequences less immunogenic to humans) according to the methods described above in Example 5, are set out in SEQ ID NO: 69 (the kappa chain of he1 and he2), SEQ ID NO: 70 (the gamma chain of he1), SEQ ID NO: 71 (the gamma chain of he2 and he3) and SEQ ID NO: 72 (the kappa chain of he3)

The chimeric H65 antibody fragments were conjugated to the Gel_{C247} analog in the same manner as

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described below for conjugation of human engineered Fab and Fab' fragments to Gel$_{C247}$ and Gel$_{A50(C44)}$.

     (i) <u>hel Fab-Gel$_{C247}$</u>

     The hel Fab was dialyzed into 25 mM TEOA buffer, 250 mM NaCl, pH 8 and then concentrated to 6.8 mg/mL prior to derivitization with the M2IT crosslinker. For the linker reaction, M2IT was used at 20-fold molar excess, in the presence of 2.5 mM DTNB. The reaction was allowed to proceed for 30 minutes at room temperature, then desalted on GF05 (gel filtration resin) and equilibrated in 0.1 M Na phosphate, 0.2M NaCl, pH 7.5. A linker number of 1.8 linkers per Fab was calculated based on the DTNB assay. The hel Fab-M2IT-TNB was concentrated to 3.7 mg/mL prior to conjugation with Gel$_{C247}$.

     Gel$_{C247}$ at 12.8 mg/mL in 10 mM Na phosphate, 0.3M NaCl, was treated with 1 mM DTT, 0.5 mM EDTA for 20 minutes at room temperature to expose a reactive sulfhydryl for conjugation and then was desalted on GF05 and equilibrated in 0.1 M Na phosphate, 0.2 M NaCl, pH 7.5. Free thiol content was determined to be 0.74 moles of free SH per mole of Gel$_{C247}$ using the DTNB assay. The gelonin was concentrated to 8.3 mg/mL prior to conjugation with activated antibody.

     The conjugation reaction between the free thiol on Gel$_{C247}$ and the derivitized hel Fab-M2IT-TNB, conditions were as follows. A 5-fold excess of the gelonin analog was added to activated hel Fab-M2IT-TNB (both proteins were in 0.1M Na phosphate, 0.2M NaCl, pH7.5) and the reaction mixture was incubated for 3.5 hours at room temperature and then overnight at 4°C. Following conjugation, untreated M2IT linkers were quenched with 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction solution was loaded onto a gel filtration column (G-75) equilibrated with 10 mM Tris, 150 mM NaCl, pH 7. The first peak off this column was diluted to 30 mM NaCl

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with 10 mM Tris, pH7 and loaded on Blue Toyopearl®. The
product was eluted with 10 mM Tris, 0.5 M NaCl, pH 7.5.

(ii) hel Fab'-Gel$_{C247}$

Similarly, the H65 hel Fab' fragment was dialyzed
into 25 mM TEOA buffer, 400 mM NaCl, pH 8 at 2.9 mg/mL
prior to derivitization with the M2IT crosslinker. For the
linker reaction, M2IT was used at 20-fold molar excess, in
-the presence of 2.5 mM DTNB. The reaction was allowed to
proceed for 1 hour at room temperature then it was desalted
on GF05 (gel filtration resin) and equilibrated in 0.1 M Na
phosphate, 0.2 M NaCl, pH 7.5. A linker number of 1.6
linkers per Fab' was calculated based on the DTNB assay.
The hel Fab'-M2IT-TNB was concentrated to 3.7 mg/mL prior
to conjugation with Gel$_{C247}$

The Gel$_{C247}$ at 77 mg/mL was diluted with 10 mM Na
phosphate, 0.1 M NaCl to a concentration of 5 mg/mL,
treated with 1 mM DTT, 0.5 mM EDTA for 30 minutes at room
temperature to expose a free thiol for conjugation and then
was desalted on GF05 and equilibrated in 0.1 M Na
phosphate, 0.2 M NaCl, pH 7.5. Free thiol content was
determined to be 1.48 moles of free SH per mole of Gel$_{C247}$
using the DTNB assay. The Gel$_{C247}$ was concentrated to 10
mg/mL prior to conjugation with activated hel Fab'-M2IT-
TNB.

For the reaction between the free thiol on Gel$_{C247}$
and the derivitized hel Fab'-M2IT-TNB, conditions were as
follows. A 5.7-fold molar excess of gelonin was added to
activated   hel   Fab'-M2IT-TNB   and   the   final   salt
concentration was adjusted to 0.25 M. The reaction mix was
incubated for 1.5 hours at room temperature and then over
the weekend at 4°C. Following conjugation, unreacted M2IT
linkers were quenched with 1:1 mole cysteamine to linker
for 15 minutes at room temperature. The quenched reaction
solution was loaded onto a gel filtration column (AcA54)
equilibrated with 10 mM Tris, 250 mM NaCl, pH 7.5. The
first peak off this column was diluted to 20 mM NaCl with

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10 mM Tris, pH 7 and loaded on Blue Toyopearl® which was equilibrated in 10 mM Tris, 20 mM NaCl, pH 7. The column was then washed with 10 mM Tris, 30 mM Nacl, pH 7.5. The product was eluted with 10 mM Tris, 1 M NaCl, pH 7.5.

5   (iii) he2-Fab Gel$_{A50(C44)}$

The he2-Fab was dialyzed overnight into 25 mM TEOA, 0.25 M NaCl, pH 8 buffer and then concentrated to 13.3 mg/mL prior to derivitization with the M2IT crosslinker. For the linker reaction, M2IT was used in a
10   20-fold molar excess in the presence of 2.5 mM DTNB. The reaction was allowed to proceed for 20 minutes at room temperature and was then desalted on a GF05-LS (gel filtration) column, equilibrated in 0.1 M Na phosphate, 0.2 M NaCl with 0.02% Na azide. A linker number of 1.7 linkers
15   per Fab-M2IT-TNB was calculated based on the DTNB assay. After derivitization and gel filtration, the he2-Fab concentration was 5.2 mg/mL.

Gel$_{A50(C44)}$ at 8.33 mg/mL in 10 mM Na phosphate, pH 7.2 was treated with 5 mM DTT and 0.5 mM EDTA for 30
20   minutes at room temperature to expose a reactive thiol for conjugation and then was desalted on GF05-LS resin equilibrated in 0.1 M Na phosphate, 0.1 M NaCl with 0.5 mM EDTA plus 0.02% Na azide, pH 7.5. Free thiol content was determined to be 0.83 moles of free SH per mole of Gel$_{A50(C44)}$
25   using the DTNB assay. The gelonin was concentrated to 11.4 mg/mL prior to conjugation with activated he2-Fab.

The conjugation reaction conditions between the free thiol on Gel$_{A50(C44)}$ and the derivitized he2-Fab-M2IT-TNB were as follows. A 3-fold excess of the gelonin analog was
30   added to activated he2-Fab-M2IT-TNB (both proteins were in 0.1 M Na phosphate, 0.1 M NaCl, pH 7.5 but the gelonin solution contained 0.5 mM EDTA as well). The reaction mixture was concentrated to half its original volume, then the mixture was incubated for 4 hours at room temperature
35   followed by 72 hours at 4°C. Following the incubation

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period the efficiency of conjugation was estimated at 70-75% by examination of SDS PAGE.

Following conjugation the excess M2IT linkers
5   were quenched by incubation with 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction as loaded onto a gel filtration column (G-75) equilibrated in 10 mM Tris, 0.15 M NaCl, pH 7. The first peak off this column was diluted to 30 mM NaCl with 10 mM Tris, pH 7 and loaded onto a Blue Toyopearl® (TosoHaas)
10   column. The product was eluted with 10 mM Tris, 1 M NaCl, pH 7.5.

(iv) he3-Fab Gel$_{A50(C44)}$

Similarly, the he3-Fab was dialyzed overnight into 25 mM TEOA, 0.25 M NaCl, pH 8 buffer and then
15   concentrated to 5 mg/mL prior to derivitization with the M2IT crosslinker. For the linker reaction, M2IT was used in a 10-fold molar excess in the presence of 2.5 mM DTNB. The reaction was allowed to proceed for 45 minutes at room temperature and was then desalted on a GF05-LS (gel
20   filtration) column, equilibrated in 0.1 M Na phosphate, 0.2 M NaCl with 0.02% Na azide. A linker number of 1 M2IT per Fab-M2IT-TNB was calculated based on the DTNB assay. After derivitization and gel filtration, the he3-Fab concentration was 5.3 mg/mL.

25   Gel$_{A50(C44)}$ at 7.8 mg/mL in 0.1 M Na phosphate, 0.1 M NaCl, pH 7.5 was treated with 1.5 mM DTT and 1 mM EDTA for 30 minutes at room temperature to expose a reactive thiol for conjugation and then was desalted on GF05-LS resin equilibrated in 0.1 M Na phosphate, 0.1 M NaCl plus
30   0.02% Na azide, pH 7.5. Free thiol content was determined to be 0.66 moles of free SH per mole of Gel$_{A50(C44)}$ using the DTNB assay. The gelonin was concentrated to 5.2 mg/mL prior to conjugation with activated he3-Fab.

The conjugation reaction conditions between the
35   free thiol on Gel$_{A50(C44)}$ and the derivitized he3-Fab-M2IT-TNB were as follows. A 5-fold excess of the gelonin analog was

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added to activated he3-Fab-M2IT-TNB (both proteins were in 0.1 M Na phosphate 0.1 M NaCl, pH 7.5). The reaction mixture was incubated for 2 hours at room temperature followed by 72 hours at 4°C. Following the incubated period the efficiency of conjugation was estimated at 70-75% by examination of SDS PAGE.

Following conjugation, the excess M2IT linkers were quenched by incubation with 1:1 mole cysteamine to linker for 15 minutes at room temperature. The quenched reaction was loaded onto a GammaBind G (immobilized protein G affinity resin, obtained from Genex, Gaithersburg, Maryland) equilibrated in 10 mM Na phosphate, 0.15 M NaCl, pH 7. It was eluted with 0.5 M NaOAc, pH 3 and neutralized with Tris. It was dialyzed into 10 mM Tris, 0.15 M NaCl, pH 7 overnight, then diluted to 30 mM NaCl with 10 mM Tris, pH 7 and loaded onto a blue Toyopearl® (TosoHaas) column. The product was eluted with 10 mM Tris, 1 M NaCl, pH 7.5

Example 8

Whole Cell Kill Assays

Immunoconjugates prepared with gelonin and gelonin analogs were tested for cytotoxicity against an acute lymphoblastoid leukemia T cell line (HSB2 cells) and against human peripheral blood mononuclear cells (PBMCs). Immunoconjugates of ricin A-chain with H65 antibody (H65-RTA) and antibody fragments were also tested. The ricin A-chain (RTA) as well as the H65-RTA immunoconjugates were prepared and purified according to methods described in U.S. Patent Application Serial No. 07/306,433, supra and in International Publication No. WO 89/06968, supra.

Briefly, HSB2 cells were incubated with immunotoxin and the inhibition of protein synthesis in the presence of immunotoxin was measured relative to untreated control cells. The standard immunoconjugates H65-RTA (H65 derivitized with SPDP linked to RTA), H65-Gelonin and H65-rGelonin, H65 fragment immunoconjugate, and gelonin immunoconjugate samples were diluted with RPMI without

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leucine at half-log concentrations ranging from 2000 to 0.632 ng/ml. All dilutions were added in triplicate to wells of microtiter plates containing 1×10^5 HSB2 cells per well. HSB2 plates were incubated for 20 hours at 37°C and then pulsed with ^{3}H-Leu for 4 hours before harvesting. Samples were counted on the Inotec Trace 96 cascade ionization counter. By comparison with an untreated sample, the picomolar concentration (pM) of immunotoxin which resulted in a 50% inhibition of protein synthesis (IC_{50}) was calculated. In order to normalize for conjugates containing differing amounts of toxin or toxin analog, the cytotoxicity data were converted to picomolar toxin (pM T) by multiplying the conjugate IC_{50} (in pM) by the toxin/antibody ratio which is unique to each conjugate preparation.

The PMBC assays were performed as described by Fishwild et *al.*, *Clin. and Exp. Immunol.*, *86*:506-513 (1991) and involved the incubation of immunoconjugates with PBMCs for a total of 90 hours. During the final 16 hours of incubation, ^{3}H-thymidine was added; upon completion, immunoconjugate-induced inhibition of DNA synthesis was quantified. The activities of the H65 and chimeric H65 antibody conjugates against HSB2 cells and PBMC cells are listed in Table 2 below.

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Table 2

IC_{50} (pM T)

Conjugate	HSB2 Cells	PBMCs
H65-RTA	143	459
H65-(M2IT)-S-S-(M2IT)-Gelonin	1770	81
H65-(M2IT)-S-S-(M2IT)-rGelonin	276	75
H65-(M2IT)-S-S-Gel$_{C10}$	140	28
H65-(M2IT)-S-S-Gel$_{A50(C44)}$	99	51
H65-(M2IT)-S-S-Gel$_{C239}$	2328	180
H65-(M2IT)-S-S-Gel$_{C244}$	>5000	>2700
H65-(M2IT)-S-S-Gel$_{C247}$	41	35
H65-(M2IT)-S-S-Gel$_{C248}$	440	203
cH65-RTA$_{30}$	60	400
cH65-(M2IT)-S-S-(M2IT)-Gelonin	1770	140
cH65-(M2IT)-S-S-(M2IT)-rGelonin	153	120
cH65-(M2IT)-S-S-Gel$_{C239}$	>7000	290
cH65-(M2IT)-S-S-Gel$_{C247}$	34	60
cH65-(M2IT)-S-S-Gel$_{C248}$	238	860
H65-(M2IT)-S-S-Gel$_{A44(C50)}$	338	ND[*]
H65-(M2IT)-S-S-Gel$_{C247A44A50}$	71	ND[*]

[*] -- Not determined.

Against HSB2 cells, many of the gelonin analog immunoconjugates were significantly more potent than conjugates prepared with native gelonin or recombinant, unmodified gelonin, both in terms of a low IC_{50} value, but also in terms of a greater extent of cell kill. Against human PBMCs, the gelonin analog conjugates were at least as active as native and recombinant gelonin conjugates. Importantly, however, some of the conjugates (for example, Gel$_{C10}$, Gel$_{A50(C44)}$ and Gel$_{C247}$) exhibited an enhanced potency against PBMCs compared to native and recombinant gelonin conjugates, and also exhibited an enhanced level of cell kill.

The activities of the H65 antibody fragment conjugates against HSB2 cells and PBMC cells are listed in Tables 3 and 4 below, wherein extent of kill in Table 3

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refers to the percentage of protein synthesis inhibited in HSB2 cells at the highest immunotoxin concentration tested (1 μg/ml).

Table 3

IC$_{50}$ (pM T)

Conjugate	HSB2 Cells	PBMCs
cH65Fab'-RTA 30	530	1800
cH65Fab'-rGelonin	135	160
cH65Fab'-Gel$_{C247}$	48	64
cH65F(ab')$_2$-RTA 30	33	57
cH65F(ab')$_2$-rGelonin	55	34
cH65F(ab')$_2$-Gel$_{C247}$	23	20
cH65F(ab')$_2$-Gel$_{C248}$	181	95

Table 4

IC$_{50}$ (pM T)

Conjugate	HSB2 Cells	Extent of Kill
he1 Fab'-Gel$_{C247}$	57.7	93%
he1 Fab-Gel$_{C247}$	180.0	94%
he2-Fab-Gel$_{A50(C44)}$	363.0	91%
he3-Fab-Gel$_{A50(C44)}$	191.0	93%
cH65Fab'-Gel$_{C247}$	47.5	93%
cH65F(ab')$_2$-rGelonin	45.4	85%
cH65F(ab')$_2$-Gel$_{C247}$	77.5	83%
cH65F(ab')$_2$-Gel$_{C247}$	23.2	85%

The data in Table 3 show that monovalent (Fab or Fab') fragments conjugated to various forms of gelonin are more potent than RTA conjugates. Table 4 shows that the human-engineered gelonin-Fab conjugates exhibit a very high degree of extent of kill.

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Example 9

Properties Of Gelonin Immunoconjugates

A. Solubility

Recombinant gelonin and the gelonin analogs
5   exhibited enhanced solubility in comparison to both native
gelonin and RTA30. In addition, recombinant gelonin and
gelonin analog immunoconjugates exhibited enhanced
solubility relative to immunoconjugates prepared with
native gelonin and RTA30. This enhanced solubility was
10   particularly noteworthy for recombinant gelonin and analog
conjugates prepared with chimeric Fab fragments.

B. Disulfide Bond Stability Assay

The stability of the disulfide bond linking a RIP
to a targeting molecule (such as an antibody) is known to
15   influence the lifespan of immunoconjugates *in vivo* [See
Thorpe *et al.*, *Cancer Res.*, *47*:5924-5931 (1987),
incorporated by reference herein]. For example, conjugates
in which the disulfide bond is easily broken by reduction
in vitro are less stable and less efficacious in animal
20   models [See Thorpe *et al.*, *Cancer Res.*, *48*:6396-6403
(1988), incorporated by reference herein].

Immunoconjugates prepared with native gelonin,
recombinant gelonin and gelonin analogs were therefore
examined in an *in vitro* disulfide bond stability assay
25   similar to that described in Wawrzynczak *et al.*, *Cancer
Res.*, *50*:7519-7526 (1990), incorporated by reference
herein. Conjugates were incubated with increasing
concentrations of glutathione for 1 hour at 37°C and, after
terminating the reaction with iodoacetamide, the amount of
30   RIP released was quantitated by size-exclusion HPLC on a
TosoHaas TSK-G2000SW column.

By comparison with the amount of RIP released by
high concentrations of 2-mercaptoethanol (to determine 100%
release), the concentration of glutathione required to
35   release 50% of the RIP (the RC_{50}) was calculated. The

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results of assays for H65 antibody conjugates are set out
in Table 5 below.

Table 5

Conjugate	RC_{50} (mM)
H65-RTA 30	3.2
H65-(M2IT)-S-S-(M2IT)-gelonin	11.1
H65-(M2IT)-S-S-(M2IT)-rGelonin	3.0
H65-(M2IT)-S-S-Gel$_{C10}$	2.5
H65-(M2IT)-S-S-Gel$_{A50(C44)}$	0.6
H65-(M2IT)-S-S-Gel$_{C239}$	774.0
H65-(M2IT)-S-S-Gel$_{C244}$	1.2
H65-(M2IT)-S-S-Gel$_{C247}$	0.1
H65-(M2IT)-S-S-Gel$_{C248}$	0.4
cH65-RTA 30	2.50
cH65-(M2IT)-S-S-(M2IT)-rGelonin	2.39
cH65-(M2IT)-S-S-Gel$_{C247}$	0.11
cH65-(M2IT)-S-S-Gel$_{C248}$	0.32
H65-(M2IT)-S-S-Gel$_{A44(C50)}$	9.2
H65-(M2IT)-S-S-Gel$_{C247A44A50}$	0.3

The foregoing results indicate that the stability of the
bonds between the different gelonin proteins and H65
antibody varied greatly. With the exception of Gel$_{C10}$ and
Gel$_{C239}$, most of the gelonin analogs resulted in conjugates
with linkages that were somewhat less stable in the *in
vitro* assay than the dual-linker chemical conjugate. The
stability of the Gel$_{C239}$ analog, however, was particularly
enhanced.

The results of the assay for H65 antibody
fragment conjugates are set out in Table 6 below.

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Table 6

Conjugate	RC_{50} (mM)
hel Fab'-Gel$_{C247}$	0.07
cFab'-Gelonin	1.27
cFab'-Gel$_{C247}$	0.08
cF(ab')$_2$-RTA 30	1.74
cF(ab')$_2$-rGelonin	2.30
cF(ab')$_2$-Gel$_{C247}$ 0.09	
cF(ab')$_2$-Gel$_{C248}$ 0.32	
he2-Fab-Gel$_{A50(C44)}$	0.46
he3-Fab-Gel$_{A50(C44)}$	0.58

From the RC_{50} results presented in Tables 5 and 6, it appears that the particular RIP analog component of each immunotoxin dictates the stability of the immunotoxin disulfide bond *in vitro*.

Example 10
Pharmacokinetics Of Conjugates To H65 Antibody

The pharmacokinetics of gelonin analogs Gel$_{C247}$, Gel$_{A50(C44)}$, and Gel$_{C10}$ linked to whole H65 antibody was investigated in rats. An IV bolus of 0.1 mg/kg of ^{125}I-labelled immunoconjugate H65-(M2IT)-S-S-Gel$_{C247}$, H65-(M2IT)-S-S-Gel$_{A50(C44)}$ or H65-(M2IT)-S-S-Gel$_{C10}$ was administered to male Sprague-Dawley rats weighing 134-148 grams. Serum samples were collected from the rats at 3, 15, 30 and 45 minutes, and at 1.5, 2, 4, 6, 8, 18, 24, 48, 72, and 96 hours. Radioactivity (cpm/ml) of each sample was measured, and SDS-PAGE was performed to determine the fraction of radioactivity associated with whole immunoconjugate. Immunoconjugate-associated serum radioactivity was analyzed using the computer program PCNONLIN (SCI Software, Lexington, Kentucky). Table 7 below lists the pharmacokinetic parameters of the immunoconjugates. In that table, the standard error for each value is indicated and a one way analysis of variance is presented, IC is the immunoconjugate (specified by the abbreviation for the

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gelonin variant that is part of the immunoconjugate), n is
the number of animals in the study, Vc is the central
volume of distribution, Cl is the clearance, MRT is the
total body mean residence time, Alpha is the α half-life
5   and Beta is the β half-life of the immunoconjugate.

Table 7

IC	Vc (ml/kg)	Cl (ml/hr/kg)	MRT (hours)	Alpha (hours)	Beta (hours)
H65 Gel$_{C247}$ n=32	65.3 ± 3.4	11.0 ± 0.4	16.5 ± 1.9	2.3 ± 0.2	20.5 ± 3.0
H65 Gel$_{A50(C44)}$ n=38	61.9 ± 2.4	4.1 ± 0.1	22.7 ± 0.7	3.0 ± 0.7	17.8 ± 0.8
H65 Gel$_{C10}$ n=45	59.2 ± 1.3	2.5 ± 0.04	42.7 ± 1.1	3.3 ± 0.3	32.9 ± 1.1
p-value	0.176	<0.0001	<0.0001	0.303	<0.0001

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The Gel_{C247} immunoconjugate was found to have α and β half lives of 2.3 and 20 hours, with a total mean residence time of 17 hours. The 72 and 96 hour time points were excluded from analysis because of the poor resolution of immunoconjugate associated radioactivity on the SDS-PAGE gel for these serum samples.

Because *in vitro* studies suggested that the Gel_{C10} immunoconjugate had greater disulfide bond stability, it was anticipated that its half lives *in vivo* would be longer relative to the cys_{247} form of the immunoconjugate. The β half life of the immunoconjugate was about 33 hours compared to 20 hours for the Gel_{C247} conjugate. The total mean residence time was also much greater for the Gel_{C10} immunoconjugate (42 hours versus 42 hours for the Gel_{247} conjugate). In addition, the clearance of the Gel_{C10} immunoconjugate was 2.5 ml/hr/kg, about four times less than that of the Gel_{C247} immunoconjugate (11 ml/hr/kg). As also predicted from the *in vitro* disulfide stability data, the clearance of the $Gel_{A50(C44)}$ immunoconjugate was intermediate between those of the Gel_{C10} and Gel_{C247} immunoconjugates.

Based on these studies, the Gel_{C10} analog conjugated to H65 antibody has greater *in vivo* stability than the $Gel_{A50(C44)}$ and Gel_{C247} analogs conjugated to H65 antibody (as determined by the longer mean residence time and clearance rates), although the properties of the $Gel_{A50(C44)}$ immunoconjugate more closely resembled those of the Gel_{C10} immunoconjugate than the Gel_{C247} immunoconjugate.

Example 11

Pharmacokinetics Of Conjugates To H65 Antibody Fragments

The pharmacokinetics of Gel_{C247} and $Gel_{A50(C44)}$ analogs linked to human engineered H65 Fab fragments were also investigated in rats. An IV bolus of 0.1 mg/kg of ^{125}I-labelled he1 H65 Fab-Gel_{C247}, he2 H65 Fab-$Gel_{A50(C44)}$ or he3 H65 Fab-$Gel_{A50(C44)}$ was administered to male Sprague-Dawley rats weighing 150-180 grams. Serum samples were collected at 3,

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5, 15, 20, 30, and 40 minutes, and 1, 1.5, 3, 6, 8, 18, 24, 32, 48, and 72 hours, and were analyzed by ELISA using rabbit anti-Gelonin antibody as the capture antibody and biotin-labelled goat anti-human kappa light chain antibody

5   as the secondary antibody.   Results of the analysis are presented in Table 8 below.   In the table, the standard error for each value is shown, and IC is the immunoconjugate, n is the number of animals in the study, Vc is the central volume of distribution, Vss is the steady

10   state volume of distribution, Cl is the clearance, MRT is the total body mean residence time, Alpha is the α half-life and Beta is the β half-life of the indicated conjugate.

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Table 8

IC	Vc (ml/kg)	Vss (ml/hr/kg)	Cl (ml/hr/kg)	MRT (hours)	Alpha (hours)	Beta (hours)
he1 Gel$_{C247}$ n=27	48 ± 3	133 ± 7	62 ± 3	2.1 ± 0.1	0.33 ± 0.03	3.0 fixed
he2 Gel$_{A50(C44)}$ n=28	54 ± 5	141 ± 8	53 ± 3	2.7 ± 0.2	0.37 ± 0.04	3.1 fixed
he3 Gel$_{A50(C44)}$ n=33	77 ± 6	140 ± 20	57 ± 3	2.5 ± 0.4	0.58 ± 0.11	3.0 ± 1.0

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Comparing the three immunoconjugates, the pharmacokinetics of he1 H65 Fab-Gel$_{C247}$, he2 H65 Fab-Gel$_{A50(C44)}$ and he3-Fab-Gel$_{A50(C44)}$ were very similar, having similar alpha and beta half-lives, mean residence times, and clearance, particularly when comparing parameters obtained from the ELISA assayed curves. This is in contrast to their whole antibody immunoconjugate counterparts, where the clearance of Gel$_{C247}$ immunoconjugate (11 ml/kg/hr) was three-fold greater than that of Gel$_{A50(C44)}$ immunoconjugate (4 ml/kg/hr). This suggests that cleavage of the disulfide bond linking the Fab fragment and gelonin is not as important for the serum clearance of Fab immunoconjugates as for whole antibody immunoconjugates.

Example 12

Immunogenicity Of Immunoconjugates

Outbred Swiss/Webster mice were injected repeatedly (0.2 mg/kg each injection) with murine H65 antibody conjugates prepared with RTA, RTA30 and recombinant gelonin. The cycle was such that each animal was injected on days 1 and 2, and then the injections were repeated 28 and 29 days later. The animals received 5 such cycles of injections. One week and three weeks following each series of injections, blood was collected and the amount of anti-RIP antibodies present was determined by ELISA; peak titers for each cycle are shown in Table 9. RTA and RTA30 generated strong responses which began immediately following the first cycle of injections and remained high throughout the experiment. In contrast, no immune response was detected for the gelonin conjugate, even after 5 cycles of injections. When the conjugates were mixed with Complete Freund Adjuvant and injected i.p. into mice, anti-RTA and RTA-30 antibodies were readily detected after several weeks. These data indicate that anti-gelonin antibodies, if generated, would have been detected by the ELISA assay, and suggest that recombinant

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gelonin may be much less immunogenic in animals than is RTA.

Table 9

Cycle	H65-RTA	H65-RTA30	H65-rGel
Prebleed	100	100	100
Cycle 1	168	117	100
Cycle 2	4208	1008	100
Cycle 3	7468	3586	100
Cycle 4	5707	3936	100
Cycle 5	4042	2505	100

Example 13

In vivo Efficacy Of Immunoconjugates

A human peripheral blood lymphocyte (PBL)-reconstituted, severe combined immunodeficient mouse model was utilized to evaluate the *in vivo* efficacy of various immunoconjugates comprising the gelonin analogs Gel_{C247} and $Gel_{A50(C44)}$. Immunoconjugates were tested for the capacity to deplete human blood cells expressing the CD5 antigen.

A.   Human PBL Donors And Cell Isolation

Human peripheral blood cells were obtained from lymphapheresis samples (HemaCare Corporation, Sherman Oaks, CA) or venous blood samples (Stanford University Blood Bank, Palo Alto, CA) collected from healthy donors. Blood cells were enriched for PBLs using Ficoll-Hypaque density gradient centrifugation (Ficoll-Paque®; Pharmacia, Piscataway, New Jersey) and subsequently washed 4 times with PBS. Residual erythrocytes were lysed with RBC lysing buffer (16 μM ammonium chloride, 1 mM potassium bicarbonate, 12.5 μM EDTA) during the second wash. Cell viability in the final suspension was >95% as assessed by trypan blue dye exclusion.

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B. Animals And Human PBL Transfer

        CB.17 *scid/scid* (SCID) mice were purchased from
Taconic (Germantown, New York) or were bred under sterile
conditions in a specific pathogen-free animal facility
5   (original breeding pairs were obtained from Hana Biologics,
Alameda, California). Animals were housed in filter-top
cages and were not administered prophylactic antibiotic
treatment. Cages, bedding, food and water were autoclaved
before use. All manipulations with animals were performed
10   in a laminar flow hood.

        Untreated SCID mice were bled for determination
of mouse Ig levels. Human PBL-injected mice were bled at
various intervals for quantitation of human Ig and sIL-2R.
Blood collection was from the retro-orbital sinus into
15   heparinized tubes. Blood samples were centrifuged at 300
x g for 10 min, and plasma was collected and stored at
-70°C. Mouse and human Ig were quantified using standard
sandwich ELISAs. Briefly, flat-bottom microtiter plates
(MaxiSorp Immuno-Plates, Nunc, Roskilde, Denmark) were
20   coated overnight at 4°C with goat anti-mouse IgG+IgA+IgM
(Zymed Laboratories, Inc., South San Francisco, California)
or goat anti-human Igs (Tago, Inc., Burlingame, California)
in bicarbonate buffer, pH 9.6. Plates were blocked for 2
hours at room temperature with 1% BSA in Tris-buffered
25   saline, pH 7.5 (TBS), and then incubated at 37°C for 1 hour
with standards or samples serially-diluted in TBS/1%
BSA/0.05% Tween 20. Standards used were a monoclonal mouse
IgG2a (IND1 anti-melanoma; XOMA Corporation, Berkeley,
California) and polyclonal human Ig (Sigma Chemical Co.,
30   St. Louis, Missouri). Subsequently, plates were washed
with TBS/Tween 20 and incubated at 37°C for 1 hour with
alkaline phosphatase-conjugated goat anti-mouse IgG+IgA+IgM
or goat anti-human Igs (Caltag Laboratories, South San
Francisco, California). Detection was by measurement of
35   absorbance at 405 nm following incubation with 1 mg/ml p-
nitro-phenylphosphate (Sigma) in 10% diethanolamine buffer,
pH 9.8. Plasma from a normal BALB/c mouse was used as a

positive control in the mouse Ig ELISA. Plasma samples from naive SCID mice or normal BALB/c mice did not have detectable levels of human Ig. Human sIL-2R was quantified using an ELISA kit (Immunotech S.A., Marseille, France) as
5   per the manufacturer's instructions.

Five-to-seven week old mice with low plasma levels of mouse Ig (<10μg/ml) were preconditioned with an i.p. injection of cyclophosphamide (Sigma) at 200 mg/kg. Two days later, they were injected i.p. with 25-40 x 10^6
10 freshly-isolated human PBL suspended in 0.8 ml PBS.

C.   Immunoconjugate Treatment

SCID mice were bled at approximately 2 weeks after human PBL transplantation. Mice with undetectable (<10 pM) or low plasma levels of human sIL-2R were
15 eliminated from the study. The cut-off for exclusion of mice with detectable, but low, levels of human sIL-2R was empirically determined for each study and was generally 20 pM. The remaining mice were divided into groups and were administered vehicle or immunoconjugate as an i.v. bolus
20 (0.2 mg/kg) daily for 5 consecutive days. Animals were sacrificed 1 day after cessation of treatment for quantitation of human T cells in tissues and human sIL-2R in plasma.

D.   Collection Of Tissues And Analysis Of PBL Depletion
25 Blood was collected from the retro-orbital sinus into heparinized tubes. Mice were then killed by cervical dislocation and spleens were removed aseptically. Single cell suspensions of splenocytes were prepared in HBSS by pressing the spleens between the frosted ends of sterile
30 glass microscope slides. Collected cells were washed twice with PBS. Erythrocytes were eliminated from blood and splenocyte suspensions using RBC lysing buffer. Subsequently, cells were resuspended in PBS for enumeration. Recovered cells were then assayed for Ag
35 expression using flow cytometry.

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Two to five hundred thousand cells in 100 μl of PBS/1% BSA/0.1% sodium azide were incubated on ice for 30 min. with saturating amounts of various FITC- or phycoerythrin (PE)-conjugated Abs (Becton-Dickinson, Mountain View, CA) Abs used for staining included: HLe-1-FITC (IgG1 anti-CD45), Leu 2-FITC (IgG1 anti-CD8), Leu 3 PE (IgG1 anti-CD4), and Leu M3-PE (IgG2a anti-CD14). Cells were then washed in cold buffer and fixed in 0.37% formaldehyde in PBS. Samples were analyzed on a FACscan (Becton-Dickinson) using log amplifiers. Regions to quantify positive cells were set based on staining of cells obtained from naive SCID mice. The absolute numbers of human Ag-positive cells recovered from SCID tissues were determined by multiplying the percent positive cells by the total number of cells recovered from each tissue sample. The total number of leukocytes in blood was calculated using a theoretical blood volume of 1.4 ml/mouse. The detection limit for accurate quantitation of human cells in SCID mouse tissues was 0.05%. All statistical comparison between treatment groups were made using the Mann-Whitney U test. Treatment groups were determined to be significantly different from buffer control groups when the p value was <0.05. Results are presented in Table 10 below, wherein + indicates a significant difference from controls, - indicates an insignificant difference and NT means the conjugate was not tested. CD5 Plus (XOMA Corporation, Berkeley, California) is mouse H65 antibody chemically linked to RTA and is a positive control. OX19 Fab-Gel$_{C247}$ is a negative control immunoconjugate. The OX19 antibody (European Collection of Animal Cell Cultures #84112012) is a mouse anti-rat CD5 antibody that does not cross react with human CD5.

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Table 10

Test Article	Human T Cell Depletion	
	Spleen	Blood
CD5 Plus	+	+
cH65 F(ab')$_2$	-	-
cH65 Fab'	-	-
H65-rGEL	+	+
cH65 F(ab')$_2$-rGel	+	+
cH65 Fab'-rGel	+	+
cH65 F(ab')$_2$-Gel$_{c247}$	+	NT
cH65 Fab'-Gel$_{c247}$	+	+
he1H65 Fab'-Gel$_{c247}$	+	NT
cH65 Fab'-Gel$_{A50(C44)}$	+	+
OX19 Fab-Gel$_{c247}$	-	-

All the gelonin immunoconjugates were capable of depleting human cells in the SCID mouse model.

Example 14

Construction Of Gelonin
Immunofusions With Chimeric Antibodies

Several genetic constructs were assembled which included a natural sequence gelonin gene fused to an H65 truncated heavy chain gene (Fd or Fd'), or an H65 light chain gene (kappa). In this Example, H65 Fd, Fd', and H65 light chain refer to chimeric constructs. The H65 Fd sequence consists of the nucleotides encoding the murine H65 heavy chain variable (V), joining (J) and human IgG$_1$, constant (C) domain 1 regions, including the cysteine bound to light chain IgG$_1$ and has the carboxyl terminal sequence SCDKTHT (SEQ ID NO: 130). The H65 Fd' sequence has the H65 Fd sequence with the addition of the residues CPP from the hinge region of human IgG$_1$ heavy chain, including a cysteine residue which is bound to the other human IgG$_1$ heavy chain and its F(ab')$_2$ fragment. See Better, et al., Proc. Nat. Acad. Sci. (USA), 90: 457-461 (1993), incorporated by reference herein.

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The H65 light chain sequence consists of the
nucleotides encoding the murine H65 light chain variable
(V), joining (J), and human kappa (C_k) regions. The DNA
sequences of the V and J regions of the H65 Fd and kappa
5   fragment genes linked to the *pelB* leader can be obtained
from GenBank (Los Alamos National Laboratories, Los Alamos,
New Mexico) under Accession Nos. M90468 and M90467,
respectively. Several of the gene fusions included a
gelonin gene linked at the 5' end of an H65 Fab fragment
10 ·gene while the others included a gelonin gene linked at the
3' end of an H65 Fab fragment gene. A DNA linker encoding
a peptide segment of the *E. coli* shiga-like toxin (SLT)
(SEQ ID NO: 56), which contains two cysteine residues
participating in a disulfide bond and forming a loop that
15 includes a protease sensitive amino acid sequence) or of
rabbit muscle aldolase [(RMA) as in SEQ ID NO: 57, which
contains several potential cathepsin cleavage sites] was
inserted between the gelonin gene and the antibody gene in
the constructs. Alternatively, a direct fusion was made
20 between a gelonin gene and an H65 Fab fragment gene without
a peptide linker segment. Table 11 below sets out a
descriptive name of each gene fusion and indicates the
expression plasmid containing the gene fusion and the
section of the application in which each is designated.
25 Each plasmid also includes the Fab fragment gene (shown in
parentheses in Table 11) with which each particular gene
fusion was co-expressed. The inclusion of a cysteine from
a hinge region (Fd') allows potential formation of either
monovalent Fab' or bivalent $F(ab')_2$ forms of the expression
30 product of the gene fusion.

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Table 11

Section	Plasmid	Description
B(i)	pING3754	Gelonin::SLT::Fd' (kappa)
B(ii)	pING3757	Gelonin::SLT::kappa (Fd')
B(iii)	pING3759	Gelonin::RMA::Fd' (kappa)
B(iv)	pING3758	Gelonin::RMA::kappa (Fd')
A(i)	pING4406	Fd::SLT::Gelonin (kappa)
A(ii)	pING4407	kappa::SLT::Gelonin (Fd)
A(iii)	pING4408	Fd::RMA::Gelonin (kappa)
A(iv)	pING4410	kappa::RMA::Gelonin (Fd)
C(i)	pING3334	Gelonin::Fd (kappa)

A. Fusions Of Gelonin At The
Carboxyl-Terminus Of Antibody Genes

(i) Fd::SLT::Gelonin (kappa)

A gelonin gene fusion to the 3'-end of the H65 Fd chain with the 23 amino acid SLT linker sequence was assembled in a three piece ligation from plasmids pVK1, pING3731 (ATCC 68721) and pING4000. Plasmid pVK1 contains the Fd gene linked in-frame to the SLT linker sequence and some H65 Fd' and kappa gene modules as in pING3217, shown in Better, et al., Proc. Nat. Acad. Sci. (USA): 457-461 (1993), except that the kappa and Fd' regions are reversed. Plasmid pING3731 contains the gelonin gene, and pING4000 contains the H65 kappa and Fd' genes each linked to the pelB leader sequence under the control of the araB promoter as a dicistronic message.

Plasmid pVK1 was designed to link the 3'-end of a human IgG Fd constant region in-frame to a protease-sensitive segment of the SLT gene bounded by two cysteine residues which form an intra-chain disulfide bond. The SLT gene segment (20 amino acids from SLT bounded by cysteine residues, plus three amino acids introduced to facilitate cloning) was assembled from two oligonucleotides, SLT Linker 1 and SLT Linker 2.

SLT Linker 1   (SEQ ID NO: 73)

5' TGTCATCATCATGCATCGCGAGTTGCCAGAATGGCATCT
GATGAGTTTCCTTCTATGTGCGCAAGTACTC 3'

SLT Linker 2   (SEQ ID NO: 74)

5'   TCGAGAGTACTTGCGCACATAGAAGGAAACTCATCAGAT
GCCATTCTGGCAACTCGCGATGCATGATGATGACATGCA 3'

The two oligonucleotides were annealed and ligated into a
vector (pING3185) containing PstI and XhoI cohesive ends,
destroying the PstI site and maintaining the XhoI site.
Plasmid pING3185 contained an engineered PstI site at the
3'-end of the Fd gene, and contained an XhoI site
downstream of the Fd gene. The product of this ligation,
pVK1, contained the H65 Fd gene (fused to the pelB leader)
in frame with the SLT linker segment, and contained two
restriction sites, FspI and ScaI, at the 3'-end of the SLT
linker.

Plasmid pVK1 was digested with SauI and ScaI, and
the 217 bp fragment containing a portion of the Fd constant
domain and the entire SLT gene segment was purified by
electrophoresis on an agarose gel. pING3731 was digested
with SmaI and XhoI and the 760 bp gelonin gene was
similarly purified. Plasmid pING4000 was digested with
SauI and XhoI and the vector segment containing the entire
kappa gene and a portion of the Fd gene was also purified.
Ligation of these three DNA fragments resulted in pING4406
containing the Fd::SLT::Gelonin (kappa) gene fusion vector.

(ii)   kappa::SLT::Gelonin (Fd)

A gelonin gene fusion to the 3'-end of the H65
kappa chain with the 25 amino acid SLT linker sequence (20
amino acids from SLT bounded by cysteine residues, plus 5
amino acids introduced to facilitate cloning) was assembled
from the DNA segments in pING3731 (ATCC 68721) and
pING3713.

Plasmid pING3713 is an Fab expression vector
where the H65 Fd and kappa genes are linked in a
dicistronic transcription unit containing the SLT linker

segment cloned in-frame at the 3'-end of the kappa gene.
The plasmid was constructed as follows. In a source
plasmid containing the H65 Fd and kappa genes, an *EagI* site
was positioned at the 3'-end of the kappa gene by site
directed mutagenesis without altering the encoded amino
acid sequence.   The SLT gene segment from pVK1 was
amplified with primers SLT-*EagI*-5' and SalI for in frame
linkage to the *EagI* site at the 3'-end of the kappa gene.

    SLT-*Eag*-5' (SEQ ID NO: 75)

    5' TGTTCGGCCGCATGTCATCATCATGCATCG 3'

    SalI (SEQ ID NO: 76)

    5' AGTCATGCCCCGCGC 3'

The 140 bp PCR product was digested with *EagI* and *XhoI*, and
the 75 bp fragment containing the SLT gene segment was
cloned adjacent to the Fd and kappa genes in the source
plasmid to generate pING3713.

   For construction of gene fusion to gelonin,
pING3713 was cut with *ScaI* and *XhoI*, and the vector
fragment containing the Fd gene and kappa::SLT fusion was
purified. pING3731 was digested with *SmaI* and *XhoI* and the
DNA fragment containing the gelonin gene was also purified.
The product of the ligation of these two fragments,
pING4407, contains the Fd and kappa::SLT::gelonin genes.

   (iii) Fd::RMA::Gelonin (kappa)

   A gelonin gene fusion to the 3'-end of the H65 Fd
chain with the 21 amino acid RMA linker sequence (20 amino
acids from RMA, plus 1 amino acid introduced to facilitate
cloning) was assembled in a three piece ligation from
plasmids pSH4, pING3731 (ATCC 68721) and pING4000.

   Plasmid pSH4 contains an Fd gene linked in frame
to the RMA linker sequence. The RMA gene segment was
linked to the 3'-end of Fd by overlap extension PCR as
follows. The 3'-end (constant region) of the Fd gene was
amplified by PCR from a source plasmid with the primers
KBA-γ2 and RMAG-1. Any Fd constant region may be used

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because constant regions of all human IgG$_1$ antibodies are identical in this region.

    KBA-γ2   (SEQ ID NO: 77)

    5' TCCCGGCTGTCCTACAGT 3'

    RMAG-1   (SEQ ID NO: 78)

    5' TCCAGCCTGTCCAGATGGTGTGTGAGTTTTGTCACAA 3'

The product of this reaction was mixed with primer RMA-76, which annealed to the amplified product of the first reaction, and the mixture was amplified with primers KBA-γ2 and RMAK-2.

    RMA-76   (SEQ ID NO: 79)

    5'   CTAACTCGAGAGTACTGTATGCATGGTTCGAGATGAACA

    AAGATTCTGAGGCTGCAGCTCCAGCCTGTCCAGATGG 3'

    RMAK-2   (SEQ ID NO: 80)

    5' CTAACTCGAGAGTACTGTAT 3'

The PCR product contained a portion of the Fd constant region linked in-frame to the RMA gene segment. The product also contained a ScaI restriction site useful for in-frame fusion to a protein such as gelonin, and an XhoI site for subsequent cloning.

    This PCR product was cut with SauI and XhoI and ligated adjacent to the remainder of the Fd gene to generate pSH4.

    For assembly of the gene fusion vector containing the Fd::RMA::Gelonin, kappa genes, pSH4 was cut with SauI and ScaI and the Fd::RMA segment was purified. Plasmid pING3731 was cut with SmaI and XhoI and the 760 bp DNA fragment containing the gelonin gene was purified, and pING4000 was cut with SauI and XhoI and the vector was purified. The product of the ligation of these fragments, pING4408, contained the Fd::RMA::Gelonin and kappa genes.

(iv) kappa::RMA::Gelonin (Fd)

    A gelonin gene fusion to the 3'-end of the H65 kappa chain with the 21 amino acid RMA linker sequence was assembled in a three piece ligation from plasmids pSH6, pING4408 (see the foregoing paragraph) and pING3713.

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Plasmid pSH6 contains a kappa gene linked in-frame to the RMA linker sequence. The RMA gene segment was linked to the 3'-end of kappa by overlap extension PCR as follows. The 3'-end (constant region) of the kappa gene was amplified by PCR from a source plasmid with the primers KBA-K2 and RMAK-1.

RMAK-1   (SEQ ID NO: 81)

5' TCCAGCCTGTCCAGATGGACACTCTCCCCTGTTGAA 3'

KBA-K2   (SEQ ID NO: 82)

5' GTACAGTGGAAGGTGGAT 3'

The product of this reaction was mixed with primer RMA-76 (SEQ ID NO: 81), which annealed to the amplified product of the first reaction, and the mixture was amplified with primers KBA-K2 and RMAK-2. The PCR product contained a portion of the kappa constant region linked in-frame to the RMA gene segment. The product also contained a *Sca*I restriction site useful for in-frame fusion to a protein such as gelonin, and an *Xho*I site for subsequent cloning. This PCR product was cut with *Sst*I and *Xho*I and ligated adjacent to the remainder of the kappa gene to generate pSH6.

For assembly of the gene fusion vector containing the kappa::RMA::Gelonin and Fd genes, pSH6 was cut with *Hind*III and *Pst*I and the DNA fragment containing the kappa constant region and a portion of the RMA linker (the *Pst*I RMA linker segment contains a *Pst*I site) segment was purified. Plasmid pING4408 was cut with *Pst*I and SalI and the DNA fragment containing a segment of the RMA linker, the gelonin gene and a portion of the tetracycline resistance gene in the vector segment was purified. pING3713 was cut with SalI and *Hind*III and the vector was purified. The product of the ligation of these three fragments, pING4410, contained the kappa::RMA::Gelonin and Fd genes.

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B. Fusions Of Gelonin At The
    Amino-Terminus Of Antibody Genes

        (i) Gelonin::SLT::Fd' (kappa)

        A gelonin gene fusion to the 5'-end of the H65
Fd' chain with a 25 amino acid SLT linker sequence (20
amino acids from SLT bounded by cysteine residues, plus
five amino acids introduced to facilitate cloning) was
assembled in a three piece ligation from plasmids pING3748,
pING3217, and a PCR fragment encoding the H65 heavy chain
variable region (V_H) gene segment which is the variable
region of the Fd' gene in pING3217. Plasmid pING3748
contains the gelonin gene linked in-frame to the SLT linker
sequence, and pING3217 contains the H65 Fd' and kappa genes
in a dicistronic transcription unit.

        Plasmid pING3825 (see Example 2) was amplified
with PCR primers gelo3'-*Eag* and gelo-9 to introduce an *Eag*I
restriction site at the 3'-end of the gelonin gene by PCR
mutagenesis.

        gelo3'-*Eag* (SEQ ID NO: 83)

        5' CATGCGGCCGATTTAGGATCTTTATCGACGA 3'

The PCR product was cut with *Bcl*I and *Eag*I and the 56 bp
DNA fragment was purified. Plasmid pING3713 was cut with
*Eag*I and *Xho*I, and the 77 bp DNA fragment containing the
SLT linker was purified. The 56 bp *Bcl*I to *Eag*I fragment
and the 77 bp *Eag*I to *Xho*I fragment were ligated into
pING3825 which had been digested with *Bcl*I and *Xho*I to
generate pING3748 which contains the gelonin gene linked
in-frame to the SLT linker sequence.

        For assembly of the gene fusion vector containing
the Gelonin::SLT::Fd' and kappa genes, the H65 V_H was
amplified by PCR from pING3217 with primers H65-G1 and H65-
G2, and the product was treated with T4 polymerase followed
by digestion with *Nde*I.

        H65-G1 (SEQ ID NO: 84)

        5' AACATCCAGTTGGTGCAGTCTG 3'

        H65-G2 (SEQ ID NO: 85)

        5' GAGGAGACGGTGACCGTGGT 3'

The 176 bp fragment containing the 5'-end of the H65 heavy
chain V-region was purified. Concurrently, pING3217 was
digested with *NdeI* and *XhoI*, and the 1307 bp DNA fragment
containing a portion of the Fd' gene and all of the kappa
gene was purified.   The two fragments were ligated to
pING3748 which had been digested with *ScaI* and *XhoI* in a
three piece ligation yielding pING3754 (ATCC 69102), which
contains the Gelonin::SLT::Fd' and kappa genes.

(ii)   Gelonin::SLT::kappa (Fd')

A gelonin gene fusion to the 5'-end of the H65
kappa chain with the 25 amino acid SLT linker sequence was
assembled in a three piece ligation from plasmids pING3748
(see the foregoing section), pING4000, and a PCR fragment
encoding the H65 light chain variable region (V_L) gene
segment.

For assembly of the gene fusion vector containing
the Gelonin::SLT::kappa and Fd' genes, an H65 V_L fragment
was amplified by PCR from pING3217 with primers H65-K1 and
JK1-*HindIII*, and the product was treated with T4 polymerase
followed by digestion with *HindIII*.

H65-K1   (SEQ ID NO: 86)

5' GACATCAAGATGACCCAGT 3'

JK1-*HindIII*   (SEQ ID NO: 87)

5' GTTTGATTTCAAGCTTGGTGC 3'

The 306 bp fragment containing the light chain V-region was
purified. Concurrently, pING4000 was digested with *HindIII*
and *XhoI*, and the 1179 bp DNA fragment containing the kappa
constant region and all of the Fd' gene was purified. The
two fragments were ligated to pING3748 which had been
digested with *ScaI* and *XhoI* in a three piece ligation
yielding pING3757, which contains the Gelonin::SLT::kappa
and Fd genes.

(iii) Gelonin::RMA::Fd' (kappa)

A gelonin gene fusion to the 5'-end of the H65 Fd' chain with the 24 amino acid RMA linker sequence (20 amino acids from RMA, plus 4 amino acids introduced to facilitate cloning) was assembled in a three piece ligation from plasmids pING3755, pING3217 and a PCR fragment encoding the H65 V_H gene segment. Plasmid pING3755 contains the gelonin gene linked in-frame to the RMA linker sequence, and pING3217 contains the H65 Fd' and kappa genes in a dicistronic transcription unit.

Plasmid pING3755 was assembled to contain the gelonin gene linked to the RMA linker gene segment. The RMA linker gene segment was amplified by PCR from pSH4 with primers RMA-EagI and HINDIII-2.

RMA-EagI (SEQ ID NO: 88)

5' ACTTCGGCCGCACCATCTGGACAGGCTGGAG 3'

HINDIII-2 (SEQ ID NO: 44)

5' CGTTAGCAATTTAACTGTGAT 3'

The 198 bp PCR product was cut with EagI and HindIII, and the resulting 153 bp DNA fragment was purified. This RMA gene segment was cloned adjacent to gelonin using an PstI to EagI fragment from pING3748 and the PstI to HindIII vector fragment from pING3825. The product of this three piece ligation was pING3755.

For assembly of the gene fusion vector containing the Gelonin::RMA::Fd', kappa genes, the H65 V_H was amplified by PCR from pING3217 with primers H65-G1 (SEQ ID NO: 84) and H65-G2 (SEQ ID NO: 85), and the product was treated with T4 polymerase followed by digestion with NdeI. The 186 bp fragment containing the 5'-end of the heavy chain V-region was purified. Concurrently, pING3217 was digested with NdeI and XhoI, and the 1307 bp DNA fragment containing a portion of the Fd' gene and all of the kappa gene was purified. These two fragments were ligated to pING3755 which had been digested with ScaI and XhoI in a three piece ligation yielding pING3759 (ATCC 69104), which contains the Gelonin::RMA::Fd' and kappa genes.

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(iv) Gelonin::RMA::kappa (Fd')

A gelonin gene fusion to the 5'-end of the H65 kappa chain with the 24 amino acid RMA linker sequence was assembled in a three piece ligation from plasmids pING3755, pING4000, and a PCR fragment encoding the H65 V_L gene segment.

For assembly of the gene fusion vector containing the Gelonin::RMA::kappa and Fd' genes, an H65 V_L segment was amplified by PCR from pING3217 with primers H65K-1 (SEQ ID NO: 86) and JK1-*Hind*III, and the product was treated with T4 polymerase followed by digestion with *Hind*III. The 306 bp fragment containing the 5'-end of the light chain V-region was purified. Concurrently, pING4000 was digested with *Hind*III and *Xho*I, and the 1179 bp DNA fragment containing the kappa constant region and all of the Fd' gene was purified. These two fragments were ligated to pING3755 which had been digested with *Sca*I and *Xho*I in a three piece ligation yielding pING3758 (ATCC 69103), which contains the Gelonin::RMA::kappa and Fd' genes.

C. Direct Fusions Of Gelonin At The
Amino Terminus Of Antibody Genes

(i) Gelonin::Fd' (Kappa)

A direct gelonin gene fusion was constructed from pING3754. pING3754 was digested with *Bgl*II and *Xho*I and the vector segment was purified. Concurrently, pING3754 was digested with *Eag*I, treated with T4 polymerase, cut with *Bgl*II, and the gelonin gene segment was purified. pING3754 was also cut with *Fsp*I and *Xho*I, and the Fd and kappa gene segment was purified. These fragments were assembled in a three-piece ligation to generate pING3334, which contains a direct gene fusion of gelonin to Fd' in association with a kappa gene.

Example 15

Preparation of he3 Fab And Gelonin he3Fab Immunofusions

The sections below detail the construction of human-engineering he3Fab protein and immunofusions of gelonin to he3 Fd and kappa chains.

A. he3-Fab Expression Plasmids

The he3 heavy chain V-region was PCR-amplified from plasmid pING4621 (pING4621 is fully described above in Example 5 above), with primers H65-G3, GAGATCCAGTTGGTGCAGTCTG (SEQ ID NO: 116) and H65G2 (SEQ ID NO: 85). Amplification was carried at using vent polymerase (New England Biolabs) for 25 cycles, including a 94°C denaturation for 1 minute, annealing at 50°C for 2 minutes, and polymerization for 3 minutes at 72°C. The PCR product was treated with polynucleotide kinase and digested with *Bst*EII and the V-region DNA was purified. The purified DNA fragment was then ligated into pIC100, which had been digested with *Sst*I, treated with T4 polymerase, and cut with *Bst*EII. The resulting fragment was then ligated with the *Bst*EII fragment from pING3218 (containing Fab' genes) to make pING4623 which contained the he3 Fd gene linked to the *pel*B leader sequence.

The he3 kappa V-region was next assembled as described above in Example 5 and in co-owned, co-pending U.S. Patent Application Serial No. 07/808,464, incorporated by reference herein, using six oligonucleotide primers,

$H65k-1, AGT CGT CGA CAC GAT GGA CAT GAG GAC CCC TGC TCA GTT TCT TGG CAT CCT CCT ACT CTG GTT TCC AGG TAT CAA ATG TGA CAT CCA GAT GAC TCA GT (SEQ ID NO: 117);

HUH-K6, TCA CTT GCC GGG CGA ATC AGG ACA TTA ATA GCT ATT TAA GCT GGT TCC AGC AGA AAC CAG GGA AAG CTC CTA AGA CCC T (SEQ ID NO: 118);

HUH-K7, TGA CTC GCC CGG CAA GTG ATA GTG ACT CTG TCT CCT ACA GAT GCA GAC AGG GAA GAT GGA GAC TGA GTC ATC TGG ATG TC (SEQ ID NO: 119);

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HUH-K8, GAT CCA CTG CCA CTG AAC CTT GAT GGG ACC
CCA GAT TCC AAT CTG TTT GCA CGA TAG ATC AGG GTC TTA GGA GCT
TTC C (SEQ ID NO: 120);

HUH-K4, GGT TCA GTG GCA GTG GAT CTG GGA CAG ATT
5   ATA CTC TCA CCA TCA GCA GCC TGC AAT ATG AAG ATT TTG GAA TTT
ATT ATT G (SEQ ID NO: 121); and

HUH-K5, GTT TGA TTT CAA GCT TGG TGC CTC CAC CGA
ACG TCC ACG GAG ACT CAT CAT ACT GTT GAC AAT AAT AAA TTC CAA
AAT CTT C (SEQ ID NO: 122)

10   and amplified with primers HUK-7 (SEQ ID NO: 92) and JK1-
HindIII (SEQ ID NO: 87).

The resulting PCR product was treated with T4
polymerase, digested with HindIII, and purified.   The
purified fragment was then cloned into pIC100, which had
15   first been cut with SstI, treated with T4 polymerase, and
digested with XhoI, along with the 353 bp HindIII-XhoI
fragment encoding the kappa constant region from pING3217.
The resulting plasmid was pING4627 which contains the he3
kappa sequence linked in frame to the pelB leader.

20   Plasmid pING4628, containing the pelB-linked he3
kappa and Fd genes under transcriptional control of the
araB promoter, was assembled from pING4623 and pING4627 as
follows.

An expression vector for unrelated kappa and Fd
25   genes, pNRX-2, was first cut with SauI and EcoRI, leaving
a vector fragment which contains all the features relevant
to plasmid replication, a tetracycline resistance marker,
araB transcriptional control, and the 3' end of the Fd
constant region.   [Plasmid pNRX-2 comprises an EcoRI to
30   XhoI DNA segment from pING 3104 (described in WO 90/02569,
incorporated by reference herein).   That segment contains
the replication, resistance and transcription control
features of pING3104 and is joined to an XhoI to SauI DNA
segment   from   pING1444   (described   in   WO 89/00999,
35   incorporated by reference herein) which contains the 3' end
of an Fd constant region.]   Next pING4623 was cut with
PstI, treated with T4 polymerase, digested with SauI and

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the *pel*B::Fd gene segment was then isolated. Plasmid
pING4627 was cut with *Xho*I, treated with T4 polymerase, cut
with *Eco*RI and ligated to the *pel*B::Fd gene segment and the
pNRX-2 vector fragment to generate the he3-Fab expression

5   vector pING4628. That plasmid contains two *Xho*I sites, one
located between the kappa and Fd genes, and another 4 bp
downstream of the termination codon for the Fd gene.

A vector, pING4633, which lacks the *Xho*I site
between the kappa and Fd genes was constructed. To

10   assemble pING4633, pING4623 was cut with *Eco*RI, treated
with T4 polymerase, digested with *Sau*I. The *pel*B::kappa
gene segment was then isolated and purified. The pNRX-2
vector fragment and the *pel*B::Fd gene segment were then
ligated to the purified *pel*B::kappa gene segment to form

15   pING4633.

Both   pING4633   and   pING4628   are   bacterial
expression vectors for he3-Fab and each comprises the he3
Fd and Kappa genes which are expressed as a dicistronic
message upon induction of the host cell with L-arabinose.

20   Moreover, pING4628 contains two *Xho*I restriction sites, one
located 4bp past the Fd termination codon and one in the
intergenic region between the 3' end of the Kappa gene and
the 5' end of the Fd gene. Plasmid pING4633 lacks the *Xho*I
site in the intergenic region.

25   B.   Purification Of he3Fab

Plasmids pING4628 and pING4633 were transformed
into *E. coli* E104. Bacterial cultures were induced with
arabinose and cell-free supernatant comprising the he3Fab
was concentrated and filtered into 20 mm HEPES, pH 6.8.

30   The sample was then loaded onto a CM Spheradex column (2.5
x 3 cm), equilibrated in 20 mM HEPEs, 1.5 mM NaCl, pH 6.8.
The column was washed with the same buffer and eluted with
20 mm HEPES, 27 mM NaCl, pH 6.8. The eluate was split into
2 aliquots and each was loaded onto and eluted from a

35   protein G (Bioprocessing) column (2.5 x 2.5 cm) separately.
The protein G column was equilibrated in 20 mM HEPES, 75 MM

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NaCl, pH 6.8 and the sample was eluted with 100 mM glycine, 100 mM NaCl, pH 3.0. The two eluates were combined and diluted two times with 20 mM HEPES, 3 M ammonium sulfate, pH 6.8. The diluted eluates were loaded onto phenyl sepharose high substitution Fast Flow (Pharmacia) column (2.5 x. 3.3 cm), equilibrated n 20 mM HEPES, 1.5 M ammonium sulfate, pH 6.8. The column was then eluted with 20 mM HEPES, 0.6 M ammonium sulfate, pH 6.8.

C.   Gelonin::RMA::he3Kappa, he3Fd Fusions

Other genetic constructs were assembled which included a natural sequence gelonin gene fused to an he3-Fab via a linker.

A fusion comprising Gelonin::RMA::he3Kappa, Fd was assembled from DNA from plasmids pING3755, pING4633, and pING4628. Both pING4633 and pING4628 were assembled in a series of steps whereby the he3 heavy and light V-regions were individually linked in-frame to the *pel*B leader. The heavy and light V-regions were then placed together in a dicistronic expression vector under the control of the *ara*B promoter in a bacterial expression vector.

Assembly of the Gelonin::RMA::he3Kappa, he3Fd fusions was accomplished by constructing three DNA fragments from plasmids pING3755, pING4633, and pING4628. The first such fragment was made by digesting pING3755 with *Sca*I and *Xho*I which excises the 4bp between those sites. The resulting vector fragment was purified. The second fragment for use in constructing the above fusions was obtained from plasmid pING4633, which was cut with *Ase*I (which cuts in V_L) and *Xho*I and the resulting 1404 bp fragment, containing the 3' end segment of the Kappa and Fd genes, was purified. The third fragment, comprising the 5' end of the Kappa variable region coding sequence, was prepared from the PCR amplified V_L gene contained in pING4628 using the oligonucleotide primers, Huk-7 and jk1-*Hind*III. The resulting 322 bp PCR-amplified V_L fragment was treated with T4 polymerase, digested with *Ase*I, and the 86

bp fragments containing the 5' end of V_L was purified. The
three fragment produced above were ligated together to form
pING3764. The DNA sequence of the PCR amplified V-region
was verified by direct DNA sequencing of pING3764.

5   D.   Gelonin::SLT::he3Kappa, he3Fd Fusion
              A   Gelonin::SLT::he3Kappa, he3Fd fusion was
       constructed by ligating the pING4633 and pING4628 fragments
       described in section A immediately above with a fragment
       produced from pING3748 which contains Gelonin::SLT.   The
10   pING3748 fragment was produced using ScaI and XhoI as
       described immediately above for pING3755.   The resulting
       vector was designated pING3763.

       E.   Construction of Expression Vector Containing
            Gelonin::SLT::he3Fd, he3kappa Fusions
15   An   expression   vector   containing   the
       Gelonin::SLT::he3Fd, he3kappa fusion was constructed in two
       steps form DNA segments from plasmids pING3825, pING4628,
       pING4639, pING3217 [described in Better, et al., Proc.
       Natl. Acad. Sci. (USA), 90:457-461 (1993), incorporated by
20   reference herein], and pING4627.   pING3825 was digested
       with NcoI and XhoI, generating a 654 bp fragment containing
       the 3' end of the gelonin gene and a fragment containing
       the 5' end of the gelonin gene which were purified. Next,
       pING4639 was digested with NcoI and NdeI and the 903 bp
25   fragment containing the 3' end of the Gelonin gene, the SLT
       linker, and the 5' end of V_H which resulted was purified.
       Finally, pING4628 was cut with NdeI and XhoI, resulting in
       a 523 bp fragment containing the 3' end of the Fd gene
       which was purified.   The three fragments were then ligated
30   to form plasmid pING3765 which contains a gene encoding a
       gelonin::SLT::he3Fd fusion.
              Three vector fragments were used to assemble the
       final expression vector (containing the gelonin::SLT::he3Fd
       and he3 kappa segments).   Plasmid pING3765 was digested
35   with XhoI, treated with T4 polymerase, cut with NheI (which
       releases a 265 bp fragment encoding the tetracycline

resistent marker), and the resulting vector fragment was purified.   Plasmid pING4627, which contains the he3Kappa gene linked in frame to the *pelB* leader was used for the construction of pING4628.   Plasmid pING4627 was cut with PstI, treated with T4 polymerase, and further digested with SstI.   The resulting 726 bp fragment, containing the Kappa gene (except 40 bp at the 3' end) was purified.   Plasmid pING3217 was then cut with SstI and NheI, resulting in a 318 bp fragment containing the 3' end of the Kappa gene and downstream portion, including a portion of the tetracycline resistance gene, which was purified.   Ligation of the foregoing three fragments produced the final expression vector, pING3767.

F.   Construction Of Expression Vector
   Containing Gelonin::RMA::he3Fd Fusions

Gelonin::RMA:he3Fd, he3Kappa fusion expression vectors was constructed in two steps from plasmids pING3825, pING4628, pING3217, and pING4627.   The cloning scheme used was identical to that used to generate pING3767 except that pING4638 was substituted for pING4639.   Plasmid pING4638 differs from pING4639 as described below in Example 16.   The intermediate vector encoding the Gelonin::RMA::Fd fusion was designated pING3766 and the final expression vector was designated pING3768.

Example 16

Gelonin-Single Chain Antibody Fusions

The natural sequence gelonin gene was also fused to a single chain form of the human engineered he3 H65 variable region.   The gelonin gene was positioned at either the N-terminus or the C-terminus of the fusion gene and the SLT or RMA linker peptide was positioned between the gelonin and antibody domains to allow intracellular processing of the fusion protein with subsequent cytosolic release of gelonin.

A. Construction of Gel::RMA::SCA(V_L-V_H), Gel::SLT::SCA
(V_L-V_H), Gel::RMA::SCA(V_H-V_L), and Gel::SLT::SCA(V_H-V_L)

A single chain antibody (SCA) form of the he3 H65
variable domain was assembled from previously constructed
genes. This SCA segment consisted of the entire V and J
region of the one chain (heavy or light) linked to the
entire V and J segment of the other chain (heavy or light)
via a 15 amino acid flexible peptide: [(Gly)$_4$ Ser]$_3$. This
peptide is identical to that described in Huston *et al.*,
Proc. Natl. Acad. Sci. USA, 85:5879-5883 (1988);
Glockshuber *et al.*, *Biochemistry*, 29:1362-1367 (1990); and
Cheadle *et al.*, *Molecular Immunol.*, 29:21-30 (1992). The
SCA was assembled in two orientations: V-
J_{kappa}::[(Gly)$_4$Ser]$_3$::V-J_{Gamma} and V-J_{Gamma}::[(Gly)$_4$Ser]$_3$::V-J_{kappa}.
Each SCA segment was assembled and subsequently fused to
gelonin.

For assembly of the SCA segment V-
J_{kappa}::[(Gly)$_4$Ser]$_3$::V-J_{Gamma}, primers HUK-7 and SCFV-1 were
used to amplify a 352 bp DNA fragment containing the he3
V/J kappa sequences from pING4627 by PCR in a reaction
containing 10 mM KCl, 20 mM TRIS pH 8.8, 10 mM $(NH_4)_2SO_2$, 2mM
$MgSO_4$, 0.1% Triton X-100., 100 ng/ml BSA, 200 uM of each
dNTP, and 2 Units of Vent polymerase (New England Biolabs,
Beverley, Massachusetts) in a total volume of 100 μl.

SCFV-1   (SEQ ID NO:91)

5' CGGACCCACCTCCACCAGATCCACCGC

CACCTTTCATCTCAAGCTTGGTGC 3'

HUK-7   (SEQ ID NO: 92)

5' GACATCCAGATGACTCAGT 3'

Concurrently, primers SCFV-2 and SCFV-3 were used to
amplify a he3 heavy chain V/J gamma segment from pING4623,
generating a 400 bp fragment.

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SCFV-2   (SEQ ID NO: 93)

5' GGTGGAGGTGGGTCCGGAGGTGGAGGATCTGA
GATCCAGTTGGTGCAGT 3'

SCFV-3   (SEQ ID NO: 94)

5' TGTACTCGAGCCCATCATGAGGAGACGGTGACCGT 3'

The products from these reactions were mixed and amplified with the outside primers HUK-7 and SCFV-3. The product of this reaction was treated with T4 polymerase and then cut with XhoI. The resulting 728 bp fragment was then purified by electrophoresis on an agarose gel. This fragment was ligated into the vectors pING3755 and pING3748 (see Example 10), each digested with ScaI and XhoI. The resulting vectors pING4637 and pING4412 contain the Gelonin::RMA::SCA V-J_{kappa}::[(Gly)$_4$Ser]$_3$::V-J_{Gamma} and Gelonin::SLT::SCA V-J_{kappa}::[(Gly)$_4$Ser]$_3$::V-J_{Gamma} fusion genes, respectively. The 728 bp fragment was also ligated into pIC100 previously digested with SstI, treated with T4 polymerase and digested with XhoI, to generate pING4635. This plasmid contains the pelB leader sequence linked in-frame to the V-J_{Kappa}::[(Gly)$_4$Ser]$_3$::V-JJ_{gamma} gene. The pelB::SCA gene in pING4635 was excised as an EcoRI-XhoI restriction fragment and cloned into the bacterial expression vector to generate pING4640.

Similarly, the SCA V-J_{Gamma}::[(Gly)$_4$Ser]$_3$::V-J_{kappa} was assembled by amplification of pING4627 with primers SCFV-5 and SCFV-6 generating a 367 bp fragment containing he3 V/J kappa sequences,

SCFV-5   (SEQ ID NO: 95)

5' GGTGGAGGTGGGTCCGGAGGTGGAGGATCT
GACATCCAGATGACTCAGT 3'

SCFV-6   (SEQ ID NO: 96)

5' TGTACTCGAGCCCATCATTTCATCTCAAGCTTGGTGC 3'

and pING4623 with primers H65-G3 and SCFV-4 generating a 385 bp fragment containing he3 gamma V/J sequences by PCR with Vent polymerase.

H65-G3   (SEQ ID NO: 97)

5' GAGATCCAGTTGGTGCAGTCTG 3'

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SCFV-4   (SEQ ID NO: 98)

5' CGGACCCACCTCCACCAGATCC
ACCGCCACCTGAGGAGACGGTGACCGT 3'

The products from these reactions were mixed and amplified
with H65-G3 and SCFV-6. The 737 bp product was treated
with T4 polymerase and cut with *XhoI*. Ligation into
pING3755 and pING3748 (digested with *ScaI* and *XhoI*)
resulted in assembly of the Gelonin::RMA::SCA V-
J_{Gamma}::[(Gly)$_4$Ser]$_3$::V-J_{kappa} gene fusion in pING4638 and
Gelonin::SLT::SCA V-J_{Gamma}::[(Gly)$_4$Ser]$_3$::V-J_{kappa} gene fusion
in pING4639, respectively.

The vectors pING4637, pING4412, pING4638 and
pING4639 were each transformed into *E. coli* strain E104 and
induced with arabinose. Protein products of the predicted
molecular weight were identified by Western blot with
gelonin-specific antibodies.

B.   Construction of
      SCA(V$_L$-V$_H$)::SLT::Gelonin Vectors

The expression vector containing SCA(V$_L$-
V$_H$)::SLT::Gelonin fusions was assembled using restriction
fragments from previously-constructed plasmids pING4640
(containing SCA(V$_L$-V$_H$)) pING4407 (containing
Kappa::SLT::Gelonin, Fd), and pING3197. Plasmid pING4640
was first cut with *BspHI*, filled in with T4 polymerase in
the presence of only dCTP, treated with mung bean nuclease
(MBN) to remove the overhang and to generate a blunt end,
and cut with *EcoRI*. The resulting 849 bp fragment was
purified. The SLT-containing fragment from pING4407 was
excised by cutting with *EagI*, blunted with T4 polymerase,
cut with *XhoI*, and the approximately 850 bp fragment which
resulted was purified. The two fragments were ligated
together into pING3197, which had been treated with *EcoRI*
and *XhoI* to generate pING4642. The DNA sequence at the
BspHI-T4-MBN/*EagI* junction revealed that two of the
expected codons were missing but that the fusion protein
was in frame.

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C.   Construction of
     SCA(V_H-V_L)::SLT::Gelonin Vectors

The expression vector containing the SCA(V_H-V_L)::SLT::Gelonin fusions was assembled using DNA from plasmids pING4636, (the *E. coli* expression vector for SCA(V_H-V_L)) and pING4407. Plasmid pING4636 was cut with *BstEII* and *XhoI* and the resulting vector fragment was purified. Concurrently, pINg4636 was used as a template for PCR with primers SCFV-7, 5'TGATGCGGCCGACATCTCAAGCTTGGTGC (SEQ ID NO: 112) and H65-G13, TGATGCGGCCGACATCTCAAGCTTGGTGC3' (SEQ ID NO: 113). The amplified product was digested with *EagI* and *BstEII* and the resulting approximately 380 bp fragment was purified. Plasmid pING4407 was then cut with *EagI* and *XhoI*, resulting in an approximately 850 bp fragment, which was purified. The three above fragments were ligated together to produce pING4643.

D.   Construction of
     SCA(V_L-V_H)::RMA::Gelonin Vectors

Expression vectors containing SCA(V_L-V_H)::RMA::Gelonin fusions were assembled using DNA from pING4640, pING4408 [Example 14A(iii)], and pING3825 (Example 2C). Plasmid pING4640 was cut with *SalI* and *BstEII* and the resulting approximately 700 bp vector fragment (containing the tetracycline resistance matter) was purified. Next, pING3825 was digested with *NcoI* and *SalI*, resulting in an approximately 1344 bp fragment containing the 3' end of the gelonin gene and adjacent vector sequences. That fragment was purified. Plasmid pING4408 was then PCR amplified with oligonucleotide primers, RMA-G3 5'TCTAGGTCACCGTCTCCTCACCATCTGGACAGGCTGGA3' (SEQ ID NO: 114), and gelo-10. The resulting PCR product was cut with *BstEII* and *NcoI* to generate an approximately 180 bp fragment containing the 3' end of V_H, RMA, and the 5' end of the Gelonin gene which was purified. The above

three fragments were ligated to generate the final
expression vector, pING4644.

E.   Construction of
     SCA(V$_H$-V$_L$)::RMA::Gelonin Vectors

5   Expression   vectors   containing   SCA(V$_H$-
V$_L$)::RMA::Gelonin were constructed using DNA from pING 4636,
pING4410, and pING3825. Plasmid pING4636 was digested with
*Sal*I and *Hind*III and the resulting vector fragment was
purified. Next, pING3825 was cut with *Nco*I and *Sal*I and
10   the 1344 bp fragment which resulted contained the 3' end of
the gelonin gene and adjacent vector sequences encoding
tetracycline resistance was purified. Finally, pING4410
was   PCR   amplified   with   primers   RMA-G4,
5'TTCGAAGCTTGAGATGAAACCATCTGGACAGGCTGGA3' (SEQ ID NO: 115)
15   and gelo-10. The PCR product was cut with *Hind*III and
*Nco*I, resulting in a 180 bp fragment containing the 3'end
of V$_L$, RMA, and the 5' end of Gelonin and was purified. The
three above fragments were ligated together to generate the
final expression vector, pING4645.

20   Gelonin::SCA fusions without a cleavable linker
may be constructed by deletion of the SLT linker in
pING4412 using the restriction enzymes *Eag*I and *Fsp*I.
Digestion at these sites and religation of the plasmid
results in an in-frame deletion of the SLT sequence.

25   **Example 17**
Multivalent Immunofusions

         Multivalent forms of the immunofusions may be
constructed.

A.   Construction of (Gel::RMA::kappa, Fd')$_2$
30   and (Gel::RMA::Fd', kappa)$_2$ Expression Vectors

         Bacterial Fab expressionn vectors can result in
the production of F(ab')$_2$ if the two cysteine residues from
the human IgG1 hinge region are included into the carboxyl-
terminus of the Fd protein [Better et al., *Proc. Natl.*
35   *Acad. Sci. USA, 90*:457-461 (1993)]. To express a gelonin

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fusion protein that could form a bi-valent structure like an F(ab')$_2$, the he3 Fd' (2C) hinge region (Better *et al.*, *supra*) was cloned into the expression vector pING3764 (Example 15C) encoding the fusion protein Gel::RMA::kappa,
5   Fd.

Plasmid pING3764 was cut with *Xho*I and *Bsu*36I and the approximately 7500 bp fragment containing the immunofusion gene and vector sequences was purified. Plasmid pING4629, which encodes he3 F(ab')$_2$, was also cut
10   with *Bsu*36I and *Xho*I, and the approximately 200 bp DNA fragment containing the he3 Fd' (2C) gene segment was purified. These two DNA fragments were ligated to generate pING3775 encoding (Gel::RMA::kappa, Fd')$_2$. An expression vector encoding the fusion protein (Gel::RMA::Fd', kappa)$_2$
15   was also made.

B.   Construction of Vectors Containing **Both** Gel::RMA::Fd
       and Gel::RMA::K Fusions

In order to construct a plasmid comprising Gel::RMA::Fd and Gel::RMA::k fusions, plasmid pING3764
20   [described above in Example 15(b)] was digested with *Bsg*I and *Sau*I and a 5.7 kb vector fragment containing plasmid replication functions, Gel::RMA::k, and the 3' end of Fd was isolated and purified. Plasmid pING3768 [described above in Example 15(E)] was digested with *Sau*I and
25   partially digested with *Pst*I and a 1.5 kb fragment containing Gel::RMA::Fd was purified. Finally, pING4000 [described above in Example 14] was digested with *Bsg*I and *Pst*I, generating a 350 bp fragment containing the 3' end of the kappa gene. That fragment was purified and the 5.7 kb,
30   1.5 kb, and 350 kb fragments described above were ligated together to form pING3770, containing the gelonin::RMA::k and gelonin:RMA::Fd fusions.

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C. Construction of Vectors Containing Both Gel::SLT::Fd
    and Gel::SLT::k Fusions

           Plasmid pING3772 contains the above-entitled
fusions and was constructed as follows. Plasmid pING3763
[described above in Example 15(D)] was digested with BsgI
and SauI and a 5.7 kb fragment containing the replication
functions, the 5' end of Gel::SLT::k and the 3' end of Fd
was generated and purified. Next, plasmid pING3767
[described in Example 15(D) above] was digested with SauI
and PstI, generating a 1.5 kb fragment containing the 5'
end of the gel::SLT::Fd fusion. That fragment was purified
and pING4000 [described in Example 14 above] was digested
with BsgI and PstI. The resulting 350 bp fragment was
purified and the above-described 5.7 kb, 1.5 kb, and 350 bp
fragments were ligated to form pING3772.

D. Expression of Multivalent Fusions

           Both pING3770 and pING3772 were transformed into
E. coli (E104) cells by techniques known to those of
ordinary skill in the art and induced with arabinose.
Concentrated supernatants from the transformed cell
cultures were analyzed by Western blot analysis with rabbit
anti-gelonin antiserum. Transformants from both plasmids
generated a reactive band on the gel at the size expected
for a Fab molecule carrying two gelonins (approximately 105
kD). These results are consistent with the production of
fusion proteins comprising monovalent Fab, with both Fd and
kappa chains separately fused to gelonin.

           E. coli strains containing plasmids pING3775,
pING3770 and pING3772 were grown in fermenters and the
fusion protein products were purified. The
(Gel::RMA::kappa,Fd')₂ expressed from pING3775 was purified
as described in Better et al., supra.

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Example 18

Construction of Expression Vectors
Encoding Immunofusions Without Linkers

Expression vectors encoding direct fusions of gelonin and dicistronic he3 Fab protein or single chain antibody were constructed as follows.

A. $V_H V_L$::Gel

Plasmid pING4642 (Example 16B) which encodes the $V_L V_H$::SLT::Gel fusion protein was cut with *Fsp*I and *Nco*I, and the approximately 100 bp DNA fragment containing the 5'-end of the gelonin gene was purified. Plasmid pING4643 (Example 16C), which encodes the $V_H V_L$::SLT::Gel fusion protein, was cut with *Eag*I, treated with T4 polymerase and cut with *Pst*I. The approximately 850 bp DNA fragment encoding the $V_H V_L$ gene segment was purified. The DNA fragments from pING4642 and pING4643 were ligated into the vector DNA fragment from pING4644 (Example 16D) [THIS PLASMID CONTRIBUTES THE REST OF THE GELONIN GENE THEN?] that had been cut with *Pst*I and *Nco*I to generate pING3781, which encodes the $V_H V_L$::Gel direct gene fusion.

B. $V_L V_H$::Gel

Plasmid pING4640 which encodes the he3 SCA gene $V_L V_H$ was cut with B*sp*HI, treated with T4 polymerase in the presence of the nucleotide dCTP only, treated with mung bean nuclease to remove the remaining 5' overhang, and then cut with *Eco*RI. The approximately 800 bp DNA fragment containing the he3 $V_L V_H$ gene was then purified on an agarose gel.

Plasmid pING3781 which encodes the direct fusion $V_H V_L$::Gel was digested with *Eag*I, treated with T4 polymerase, and then digested with *Xho*I. The approximately 800 bp DNA fragment encoding the gelonin gene was then purified on an agarose gel. The two DNA fragments from pING4640 and pING3781 were ligated into the vector DNA from pING3767 which had been digested *Eco*RI and *Xho*I and purified on an agaraose gel. The resultant plasmid,

pING3348, encoded the V_LV_H::Gel fusion protein. The DNA sequence at the fusion junction was verified by direct DNA sequencing.

C. Gel::V_HV_L

The plasmid pING3755 [Example 14B(iii)], which contains the gelonin gene with an engineered EagI site at its 3'-end, was cut with EagI, treated with T4 polymerase, and digested with NcoI. The approximately 650 bp DNA fragment containing the 3'-end of the gelonin gene was purified on an agarose gel. The plasmid pING4639 (Example 16A) encoding the fusion Gel::SLT::V_HV_L was cut with XhoI and then partially digested with FspI. The approximately 730 bp DNA fragment containing all of the he3 V_HV_L gene was then purified in an agarose gel (a single FspI restriction site occurs in the V_H gene segment, and the purified he3 V_HV_L gene was separated from the incomplete gene segment which was approximately 660 bp). The two DNA fragments from pING3755 and pING4639 were ligated into the vector pING3825 that had been digested with NcoI plus XhoI and purified on an agarose gel. The plasmid pING3350 was generated which encoded the Gel::V_HV_L fusion protein. The DNA sequence at the fusion junction was verified by direct DNA sequencing.

D. Gel::V_LV_H

Plasmid pING3336 which encodes the he3 V_LV_H single chain antibody gene was cut with SstI and AseI, and the approximately 5500 bp DNA fragment containing the 3'-end of V_LV_H and downstream vector sequences was purified. (pING3336 is identical to pING4640 except that the V_LV_H gene encodes six histidine residues in frame at the carboxyl-terminus). Plasmid PING4627 (Example 15A) served as a substrate for PCR amplification of the V_H gene segment. Plasmid pING4627 was amplified with the two oligonucleotide primers HUK-7 (SEQ ID NO: 92) and JK1-HindIII (SEQ ID NO: 87), the resultant product was treated with T4 polymerase and cut with AseI, and the 86 bp DNA fragment containing

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the 5'-end of the V_L was purified. The DNa fragments from pING3336 and pING4627 were ligated to the approximately 2350 bp DNA fragment of pING3755 generated by digestion with EagI, treatment with T4 polymerase and subsequent

5   digestion with SstI. The resultant vector containing the Gel::$V_L V_H$ gene fusion was named pING4652. The DNA sequence of pING4652 was verified at ligation juctions.

E.   Gel::kappa, Fd

         The direct gene fusion which encodes Gel::kappa,

10   Fd was also assembled from DNA segments from three plasmids. Plasmid pING3764 (Example 15C) was digested with HindIII and XhoI, and the approximately 1200 bp DNA fragment encoding the 3'-end of the kappa gene and the Fd gene was purified. Plasmid pING4652, which encodes a

15   direct gene fusion of gelonin to the he3 SCA gene $V_L V_H$, was cut with BglII and HindIII, and the approximately 850 bp DNA fragment encoding the 3'-end of the gelonin gene and the V_L region of kappa was purified. The DNA fragments from pING3764 and pING4652 were ligated into the vector fragment

20   from pING3825 (Example 2C) that had been digested with BglII and XhoI to generate pING3784 encoding Gel::kappa, Fd.

F.   Gel::Fd, kappa

         Plasmid pING3768 (Example 15F), which encodes the

25   fusion protein Gel::RMA::Fd, kappa, was cut with NdeI and NheI, and the DNA segment containing the majority of the he3 Fd gene, the he3 kappa gene and a portion of the tetracycline resistance gene of the vector was purified. Plasmid pING3350, which is described in section C above,

30   was cut with NdeI and PstI, and the DNA fragment containing the 5'-end of the he3 Fd gene linked to the gelonin gene was purified. The DNA fragments from pING3350 and pING3768 were ligated into the vector fragment from pING4633 (Example 16D) that had been cut with NheI and PstI to

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generate pING3789.   Plasmid pING3789 encodes the fusion
protein Gel::Fd, kappa.

Example 19

Alternative Cathepsin Cleavable Linkers

5   The segment of rabbit muscle aldolase chosen for
the RMA linker described herein is known to contain peptide
sequences susceptible to digestion with cathepsins. Other
cathepsin-cleavable protein segments are effective targets
for intracellular cleavage, and two particular amino acid
10   sequences were included as cleavable linkers in additional
immunofusions of the invention. These are the amino acid
sequence KPAKFFRL (SEQ ID NO: 141 ("CCF") and KPAKFLRL (SEQ
ID NO: 142) ("CCL"). Two oligonucleotides were synthesized
that encode these peptide segments. Degeneracy was
15   introduced at one nucleotide position in each synthetic
primer to allow the appropriate amino acid to be encoded at
the particular amino acid position in which CCF and CCL
differ.   The   two   oligonucleotides   5′-
GGCCGCAAAGCCGGCTAAGTTCTT(A/C)CGTCTGAGT-3′ (SEQ ID NO: 143)
20   and 5′-ACTCAGACG(G/T)AAGAACTTAGCCGGCTTTGC-3′ (SEQ ID NO:
144). The oligonucleotide linkers were then used to
assemble a family of fusion gene expression vectors
encoding: Gel::CCL::kappa, Fd; Gel::CCF::kappa, Fd;
Gel::CCF::V_LV_H; and Gel::CCL::V_HV_L.

25   The CCL and CCF linkers were also included
in fusion vectors where the antigen-binding domain of the
fusion protein was at the N-terminus of the fusion to
generate expression vectors encoding immunofusions such as
V_LV_H::CCL::Gel.

30   Several of the fusion proteins with the CCL
and CCF linkers were tested for cytotoxicity on the T cell
lines HSB2 and PBMC and were comparable in activity to the
fusion proteins containing the RMA linker.

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Example 20

Expression And Purification Of Gelonin Immunofusions

A.   Expression Of Gelonin Immunofusions

Each of the gelonin gene fusions whose
5   construction is described in Example 15 was co-expressed
with its pair H65 Fab gene in arabinose-induced *E. coli*
strain E104.

Expression products of the gene fusions were
detected in the supernatant of induced cultures by ELISA.
10   Typically, a plate was coated with antibody recognizing
gelonin. Culture supernatant was applied and bound Fab was
detected with antibody recognizing human kappa coupled to
horseradish peroxidase.   H65 Fab fragment chemically
conjugated to gelonin was used a standard.   Alternative
15   ELISA protocols involving coating a plate with antibody
recognizing either the kappa or Fd or involving a detection
step with anti-human Fd rather than anti-human kappa
yielded similar results.   Only properly assembled fusion
protein containing gelonin, kappa and Fd was detected by
20   this assay. Unassociated chains were not detected.

The fusion protein produced from induced cultures
containing expression vectors pING4406, 4407, 4408, and
4410 in *E. coli* E104 accumulated at about 20-50 ng/ml. The
fusion proteins expressed upon induction of pING3754, 3334,
25   3758 and 3759 (but not pING3757) were expressed at much
higher levels, at about 100 to 500 ng/ml. A fusion protein
of about 70,000 Kd was detected in the concentrated *E. coli*
culture supernatant by immunostaining of Western blots with
either anti-human kappa or anti-gelonin antibodies.

30   The Gelonin::SLT::Fd' (kappa) fusion protein from
pING3754 (ATCC 69102) was purified from induced 10 L
fermentation broth.   The 10 L fermentation broth was
concentrated and buffer exchanged into 10mM phosphate
buffer at pH 7.0, using an S10Y10 cartridge (Amicon) and a
35   DC10 concentrator. The supernatant was purified by passing
the concentrated supernatant through a DE52 column (20 x 5
cm) equilibrated with 10 mM sodium phosphate buffer at pH

7.0.   The flow-through was then further purified and
concentrated by column chromatography on CM52 (5 x 10 cm)
in 10 mM phosphate buffer. A 0 - 0.2 M linear gradient of
NaCl was used to the elute the fusion protein, and
5   fractions containing the fusion protein were pooled and
loaded onto a Protein G column (1ml). The fusion protein
was eluted from protein G with 0.2 M sodium citrate, pH 5.5
and then 0.2 M sodium acetate, pH 4.5, and finally, 0.2 M
glycine, pH 2.5.   The Gelonin::RMA::Fd′ (kappa) and
10   Gelonin::RMA::kappa (Fd′) fusions proteins were purified
from fermentation broths by similar methods except that the
CM52 column step was eliminated, and the DE52 column was
equilibrated with 100mM sodium phosphate buffer at pH 7.0.
The fusion proteins were not purified to homogeneity.

15   Each of the three purified fusion proteins was
then assayed for activity in the RLA assay and for
cytotoxicity against the T-cell line HSB2.   (T cells
express the CD5 antigen which is recognized by H65
antibody.)   The RLA assay was performed as described in
20   Example 4 and results of the assay are presented below in
Table 12.

Table 12

Fusion Protein	IC50(pM)
rGelonin	11
Gelonin::SLT::Fd (kappa)	19
Gelonin::RMA::Fd (kappa)	28
Gelonin::RMA::kappa (Fd)	10

Two fusion proteins were tested in whole cell cytotoxicity
assays performed as described in Example 6 (Table 13). As
30   shown in Table 13, the fusion proteins were active.
Gelonin::SLT::Fd(kappa) killed two T cell lines, HSB2 and
CEM, with respective IC_{50}s 2-fold (HSB2) or 10-fold (CEM)
higher than that of the gelonin chemically linked to H65.
See Table 13 below for results wherein IC_{50} values were

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adjusted relative to the amount of fusion protein in each sample.

Table 13
IC$_{50}$ (pMT)

Fusion Protein	HSB2 Cells	CEM Cells
he3Fab-Gel$_{A50(C44)}$	165	173
Gelonin::SLT::Fd (kappa)	180	1007
Gelonin::RMA::Fd (kappa)	150	NT

These fusion protein showed similar activity on peripheral blood mononuclear cells (data not shown).

B.   Purification of Immunofusions

(i)   Immunofusions Comprising cH65Fab'

Immunofusions comprising a cH65Fab' fragment were purified from cell-free supernatants by passing the supernatant through a CM Spheradex (Sepacor) column (5cm x 3cm), equilibrated in 10 Mm Na phosphate at pH 7.0. Immunofusion proteins bind to the column and are eluted with 10 mM Na phosphate, 200 mM NaCl, pH 7.0. The eluate was diluted two-fold with 20 Mm HEPES, 3 M ammonium sulfate, pH 7.6 and loaded onto a phenyl sepharose fast flow (Pharmacia) column (2.5 x3.5 cm), equilibrated in 20 mM HEPES, 1.2 M ammonium sulfate, pH 7.0. The column was next washed with 20 mM Hepes, 1.2 M ammonium sulfate, pH 7.0 and eluted with 20 mM HEPES, 0.9 M ammonium sulfate, pH 7.0. The phenyl sepharose eluate was concentrated to a volume of 2-4 ml in an Amicon stirred cell fitted with a YM10 membrane. The concentrated sample was loaded onto an S-200 (Pharmacia) column (3.2x 38 cm), equilibrated in 10 mm Na phosphate, 150 mm NaCl, pH 7.0. The column was run in the same buffer and fractions were collected. Fractions containing the fusion protein of desired molecular weight were combined. For example, by selection of appropriate column fractions, both monovalent (gelonin-Fab') and

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bivalent (gelonin$_2$-F(ab')$_2$ forms encoded by pING3758 were purified.

(ii)   Immunofusions Comprising he3Fab

Immunofusions comprising he3Fab were purified as in the preceding section with the exception that the phenyl sepharose column was eluted with 20 mM HEPES, 1.0 M ammonium sulfate, pH 7.0.

(iii)   Immunofusions Comprising SCA

Cell-free supernatant was passed through a CM spheradex column (5 x 3 cm), equilibrated with 10 mM Na phosphate, pH 7.0. Single-chain antibody binds to the column which is then washed with 10 mM Naphosphate, 45 mM NaCl, pH 7.0. The fusion protein was then eluted with 10 mM Naphosphate, 200 mM NaCl, pH 7.0. The eluate was diluted two-fold with 20 mM HePES, 3 M ammonium sulfate, pH 7.0 and loaded onto a butyl sepharose Fast Flow (Pharmacia) column (2.5 x 4.1 cm) equilibrated in 20 mM HEPES, 1.5 M ammonium sulfate, pH 7.0. The column was then washed with 20 mM HEPES, 1.0 M ammonium sulfate, pH 7.0 and eluted with 20 mM HEPES pH 7.0. The butyl sepharose eluate was concentrated to a volume of 2-4 ml in an Amicon stirred cell fitted with a YM10 membrane. The concentrated sample was loaded onto an S-200 (Pharmacia) column (3.2 x 38 cm) equilibrated in 10 mM Na phosphate, 150 mM NaCl, pH 7.0. The column was then run in the same buffer and the fractions were collected. Some of the fractions were analyzed by SDS-PAGE to determine which fractions to pool together for the final product.

Example 21

Activity of Gelonin Immunofusions

A concern in constructing immunofusions comprising any RIP is that the targeting and enzymatic activities of the components of the fusion protein may be lost as a result of the fusion. For example, attachment of

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an RIP to the amino terminus of an antibody may affect the antigen-binding (complementarity-determining regions) of the antibody and may also result in steric hinderance at the active site. Similarly, the activity of an RIP may be

5   hindered by attachment of an antibody or antibody portion. For example, RIPs chemically conjugated to antibodies via a disulfide bridge are typically inactive in the absence of reducing agents. In order to assess the foregoing in immunofusions of the present invention, such proteins were

10   subjected to assays to determine their enzymatic, binding, and cytotoxic activities.

A.   Reticulocyte Lysate Assay

The enzymatic activity of immunofusions comprising gelonin was assayed using the reticulocyte

15   lysate assay (RLA) describe above. As noted in Example 4, the RLA assay measures the inhibition of protein synthesis in a cell-free system using endogenous globin mRNA from a rabbit red blood cell lysate. Decreased incorporation of tritiated leucine (^{3}H-Leu) was measured as a function of

20   toxin concentration. Serial log dilutions of standard toxin (the 30 kD form of ricin A-chain, abbreviated as RTA 30), native gelonin, recombinant gelonin (rGelonin or rGel) and gelonin analogs were tested over a range of 1 μg/ml to 1 pg/ml. Samples were tested in triplicate, prepared on

25   ice, incubated for 30 minutes at 37°C, and then counted on an Inotec Trace 96 cascade ionization counter. By comparison with an uninhibited sample, the picomolar concentration of toxin (pM) which corresponds to 50% inhibition of protein synthesis (IC_{50}) was calculated.

30   Representative data for various immunotoxins of the invention are shown below in Table 14 .

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Table 14

Immunotoxin	Lot No.	IC_{50} (pM)
rGel::RMA::SCA(V_H-V_L)	AB1136	12
rGel::RMA::SCA(V_L-V_H)	AB1137	18
rGel::SLT::SCA(V_H-V_L)	AB1133	26
rGel::RMA::SCA(V_L-V_H)	AB1124	33
rGel::RMA::K+Fd'(cH65Fab')	AB1122	54
rGel::SLT::K+Fd(he3Fab)	AB1160	40
rGel::RMA::K+Fd(he3Fab)	AB1149	33
rGel::RMA::Fd+K(he3Fab)	AB1163	14
rGel::Fd'+K(cH65Fab')	AB1123	45

Contrary to the expectations discussed above, gelonin immunofusions of the invention exhibit enzymatic activity which is comparable to the activities of native and recombinant gelonin shown in Example 4. This was true for fusions made with either the reducible (SLT) or non-reducible (RMA) linkers.

B.   Binding Activity of Immunofusions

Several immunofusions according to the present invention were assayed for their ability to compete with labelled antibody for binding to CD5-positive cells. The Kd of the immunofusions was estimated by three different means as shown in Table 15. The first Kd estimation (Kd_1 in Table 15) was obtained by competition with fluorescein-labelled H65 IgG for binding to MOLT-4X cells (ATCC CRL 1582) according to the procedure reported in Knebel, et al., Cytometry Suppl., 1: 68 (1987), incorporated by reference herein.

The second Kd measurement (Kd_2 in Table15) was obtained by Scatchard analysis of competition of the immunofusion with ^{125}I-cH65 IgG for binding on MOLT-4M cells as follows. A 20 μg aliquot of chimeric H65 IgG (cH65 IgG) was iodinated by exposure to 100 μl lactoperoxidase-glucose oxidase immobilized beads (Enzymobeads, BioRad), 100 μl of PBS, 1.0 mCi I^{125} (Amersham, IMS30), 50 μl of 55 mM

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b-D-glucose for 45 minutes at 23°C. The reaction was quenched by the addition of 20 μl of 105 mM sodium metabisulfite and 120 mM potassium iodine followed by centrifugation for 1 minute to pellet the beads. ^{125}I-cH65

5   IgG was purified by gel filtration using a 7 ml column of sephadex G25, eluted with PBS (137 mM NaCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.68 mM KCl at pH 7.2-7.4) plus 0.1% BSA. ^{125}I-cH65 IgG recovery and specific activity were determined by TCA precipitation.

10   Competitive binding was performed as follows: 100 μl of Molt-4M cells were washed two times in ice-cold DHB binding buffer (Dubellco's modified Eagle's medium (Gibco, 320-1965PJ), 1.0% BSA and 10 mM Hepes at pH 7.2 -7.4). Cells were resuspended in the same buffer, plated

15   into 96 v-bottomed wells (Costar) at 3×10^5 cells per well and pelleted at 4°C by centrifugation for 5 min at 1,000 rpm using a Beckman JS 4.2 rotor; 50 μl of 2X-concentrated 0.1 nM ^{125}I-cH65 IgG in DHB was then added to each well and competed with 50 μl of 2X - concentrated cH65 IgG in DHB at

20   final protein concentrations from 100 nM to 0.0017 nM. The concentrations of assayed proteins were determined by measuring absorbance (A_{280} and using an extinction coefficient of 1.0 for fusion proteins, 1.3 for Fab, and 1.22 for Fab conjugated to gelonin. Also, protein

25   concentrations were determined by BCA assay (Pierce Chemical) with bovine serum albumin as the standard. Binding was allowed to proceed at 4°C for 5 hrs and was terminated by washing cells three times with 200 μl of DHB binding buffer by centrifugation for 5 min. at 1,000 rpm.

30   All buffers and operations were at 4°C. Radioactivity was determined by solubilizing cells in 100 μl of 1.0 M NaOH and counting in a Cobra II auto gamma counter (Packard). Data from binding experiments were analyzed by the weighted nonlinear least squares curve fitting program, MacLigand,

35   a Macintosh version of the computer program "Ligand" from Munson, *Analyt. Biochem.*, 107:220 (1980), incorporated by reference herein.

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Finally, the Kd (Kd_3 in the Table) was estimated by examination of the ED_{50} values obtained from separate competition binding assays, performed as described in the previous paragraph. All three measurements are shown in Table 15 below:

Table 15

Molecule Type	Kd_1	Kd_2	Kd_3
H65 IgG	1.6	ND	ND
cH65 IgG	ND	3.0	2.5
cH65Fab'	4.0	14.0	ND
cH65Fab'-rGel$_{A50(C44)}$	3.5	13.0	ND
rGel::RMA::K+Fd' (cH65Fab')	16.0	ND	100
he3Fab	1.20	2.60	ND
he3Fab-rGel$_{A50(C44)}$	1.10	2.70	ND
rGel::RMA::K+Fd' (he3Fab)	2.60	ND	5.0
rGel::SLT::K+Fd (he3Fab)	ND	ND	30
SCA(V_L-V_B)	2.20	ND	30
rGel::RMA::SCA(V_B-V_L)	3.50	ND	20
rGel::RMA::SCA(V_L-V_B)	4.70	ND	30
SCA(V_L-V_B)	ND	ND	20
rGel::RMA::SCA(V_L-V_B)	2.30	ND	ND

ND = not determined

The results presented in Table 15 suggest that Fab and SCA antibody forms may retain substantial binding activity even when fused to an RIP.

C. Comparative Cytotoxicity Assays

Fusion proteins and immunoconjugates according to the present invention were used in a comparative cytoxicity assay. Two **types of** assays were conducted, one targeting T cell line HSB2, and the other targeting lectin-activated peripheral blood mononuclear cells (PBMC) according to procedures in Example 6. The results of the assays are presented below in Tables 16a, 16b and 16c.

Table 16a

CYTOTOXIC POTENCIES:
CHEMICAL VS. GENE-FUSED CONJUGATES

Immunotoxin	Lot #	Fusion Plasmid	IC$_{50}$, pM Toxin	HSB2 IC$_{50}$, pM Toxin	N
CD5 Plus	HF002002	---	148	24	8
H65-M-rGel$_{A50(C44)}$	999	---	68*	NA	1
cH65-MM-rGel	807	---	183*	NA	1
cH65Fab'-M-rGel$_{A50(C44)}$	941	---	99	6	2
He2Fab-M-rGel$_{A50(C44)}$	970	---	468	195	4
he3Fab-M-rGel$_{A50(C44)}$	1012/1047	---	190	70	12
he3Fab-SMCC-rGel$_{A50(C44)}$	1086	---	5,904	2,442	2
rGel::SLT::Fd'+K(1)+	AB1095	pING3754	320	25	2
rGel::SLT::Fd'+K(3)+	AB1095	pING3754	374*	NA	1
rGel::SLT::K+Fd(he3)	AB1147	pING3763	495*	NA	1
rGel::SLT::K+Fd(he3)	AB1160	pING3763	746*	NA	1
rGel::SLT::SCA(Vh-Vl)	AB1133	pING4639	422	31	5
rGel::SLT::SCA(Vl-Vh)	AB1124	pING4412	776	347	3
rGel::RMA::K+Fd'	AB1122	pING3758	1,506	1,033	2
rGel::RMA::K+Fd'	AB1141	pING3758	5,833*	NA	1
rGel::RMA::K+Fd(he3)	AB1149	pING3764	9,154*	NA	1

Immunotoxin	Lot #	PBMC			
		IC$_{50}$, pM Toxin	IC$_{50}$, pM Toxin	SD	N
rGel::RMA::K+Fd(he3)	AB1149	7,124	10,764	14,081	5
rGel::RMA::K+Fd(he3)	AB1161	1,854	2,990	3,324	3
rGel::RMA::Fd'+K	RF524(1)	1,760	1,893	1,049	5
rGel::RMA::Fd'+K	AB1121	2,090	1,664	1,553	6
rGel::RMA::Fd+K(he3)	AB1163	854	567	406	2
rGel::RMA::SCA(Vh-Vl)	AB1136	393	567	510	7
rGel::RMA::SCA(Vh-Vl)	AB1152	9,650	9,170	6,483	3
rGel::RMA::SCA(Vl-Vh)	AB1137	4,040	4,554	4,310	7
rGel::RMA::SCA(Vl-Vh)	AB1164	1,598	1,598	1,144	2
rGel::Fd'+K	AB1123	2,606	2,777	2,167	4
K::RMA::rGel+Fd	AB1140	1,545	1,545	417	2
rGel	1056	13,350	40,233	43,048	6
8B2.3Fab-m-rGel$_{A50(C44)}$	1057	12,400	13,174	14,339	11

* -- Results represent single values and not a mean value.
+ -- rGel::SLT::Fd'+k(1) and rGel::SLT::Fd'+k(3) are separate fractions from the final purification column.

Table 16c

Fusion Plasmid	Immunotoxin	H8B2 IC50, pM Toxin	n=	PBMC IC50, pM Toxin	n=
pING4644	$V_L V_H$::RMA::Gel	1933	4	1513	29
pING3784	Gel::kappa, Fd	>12,500	3	2645	7
pING3789	Gel::Fd, kappa	1212	1	3665	1
pING3348	$V_L V_H$::Gel	2158	4	1264	9
pING3350	Gel::$V_H V_L$	8056	3	2729	4
pING3775	(Gel::RMA::kappa, Fd')$_2$	175	1	44	22
pING3770	Gel::RMA::k, Gel::RMA::Fd	3548	2	519	9
pING3772	Gel::SLT::k, Gel::SLT::Fd	-	-	663	6

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The results presented in Tables 16a, 16b and 16c demonstrate that gelonin immunofusions may vary in their activity. In general, immunofusions of the invention which have IC_{50} median or mean values of less than 2000 pM Toxin display strong activity; whereas those with IC_{50} values equal to or less than 500 pM Toxin are considered highly active. In sum, the results in Tables 16a, 16b and 16c demonstrate that the optimum fusion protein for killing a particular cell line may vary depending upon the targeted cell.

Example 22

Preparation Of BRIP

BRIP possesses characteristics which make it an attractive candidate for a component of immunotoxins. BRIP is a naturally unglycosylated protein that may have reduced uptake in the liver and enhanced circulatory residence time *in vivo*. Additionally, BRIP is less toxic and less immunogenic in animals than the A-chain of ricin. Cloning of the BRIP gene and expression of recombinant BRIP in an *E. coli* expression system obviates the need to purify native BRIP directly from barley, and enables the development of analogs of BRIP which may be conjugated with an available cysteine residue for conjugation to antibodies.

A. Purification Of BRIP And Generation Of Polyclonal Antibodies To BRIP

Native BRIP was purified from pearled barley flour. Four kilograms of flour was extracted with 16 liters of extraction buffer (10 mM NaPO4, 25 mM NaCl, pH 7.2) for 20 hours at 4°C. The sediment was removed by centrifugation, and 200 ml of packed S-Sepharose (Pharmacia, Piscataway, New Jersey) was added to absorb BRIP. After mixing for 20 hours at 4°C, the resin was allowed to settle out, rinsed several times with extraction buffer and then packed into a 2.6 x 40 cm column. Once packed, the column was washed with extraction buffer (150

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ml/h) until the absorbance of the effluent approached zero.
BRIP was then eluted with a linear gradient of 0.025 to 0.3
M NaCl in extraction buffer and 5 ml fractions were
collected. BRIP-containing peaks (identified by Western

5   analysis of column fractions) were pooled, concentrated to
about 20 ml, and then chromatographed on a 2.6 x 100 cm
Sephacryl S-200HR (Pharmacia) column equilibrated in 10 mM
NaPO$_4$, 125 mM NaCl, pH 7.4 (10 ml/hr). BRIP-containing
peaks were pooled again, concentrated, and stored at -70°C.

10   The resulting purified BRIP protein had a
molecular weight of about 30,000 Daltons, based upon the
mobility of Coomassie-stained protein bands following SDS-
PAGE. The amino acid composition was consistent with that
published by Asano et al., Carlsberg Res. Comm., 49:619-626

15 (1984).

          Rabbits were immunized with purified BRIP to
generate polyclonal antisera.

B. Cloning Of The BRIP Gene

          A cDNA expression library prepared from

20 germinating barley seeds in the phage λ expression vector
λZAPII was purchased from Stratagene, La Jolla, CA.
Approximately 700,000 phage plaques were screened with
anti-BRIP polyclonal antisera and 6 immunoreactive plaques
were identified. One plaque was chosen, and the cDNA

25 contained therein was excised from λZAPII with EcoRI and
subcloned into pUC18 generating the vector pBS1. The cDNA
insert was sequenced with Sequenase (United States
Biochemical, Cleveland, Ohio). The DNA sequence of the
native BRIP gene is set out in SEQ ID NO: 12. To confirm

30 that cDNA encoded the native BRIP gene, the cDNA was
expressed in the E. coli plasmid pKK233-2 (Pharmacia).
BRIP protein was detected in IPTG-induced cells transformed
with the plasmid by Western analysis with above-described
rabbit anti-BRIP antisera.

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C. Construction Of An *E. coli* Expression
    Vector Containing The BRIP Gene

Barley cDNA containing the-BRIP gene was linked to a *pel*B leader sequence and placed under control of an araB promoter in a bacterial secretion vector.

An intermediate vector containing the BRIP gene linked to the *pel*B leader sequence was generated. Plasmid pBS1 was cut with *Nco*I, treated with Mung Bean Nuclease, cut with *Bam*HI and the 760 bp fragment corresponding to amino acids 1-256 of BRIP was purified from an agarose gel. Concurrently, a unique *Xho*I site was introduced downstream of the 3'-end of the BRIP gene in pBS1 by PCR amplification with a pUC18 vector primer (identical to the Reverse® primer sold by NEB or BRL but synthesized on a Cyclone Model 8400 DNA synthesizer) and the specific primer BRIP 3'*Xho*. The sequence of each of the primers is set out below.

Reverse (SEQ ID NO: 45)

5' AACAGCTATGACCATG 3'

BRIP 3'*Xho* (SEQ ID NO: 46)

5' TGAACTCGAGGAAAACTACCTATTTCCCAC 3'

Primer BRIP 3'*Xho* includes a portion corresponding to the last 8 bp of the BRIP gene, the termination codon and several base pairs downstream of the BRIP gene, and an additional portion that introduces a *Xho*I site in the resulting PCR fragment. The PCR reaction product was digested with *Bam*HI and *Xho*I, and an 87 bp fragment containing the 3'-end of the BRIP gene was purified on a 5% acrylamide gel. The 760 and 87 bp purified BRIP fragments were ligated in the vector pING1500 adjacent to the *pel*B leader sequence. pING1500 had previously been cut with *Sst*I, treated with T4 polymerase, cut with *Xho*I, and purified. The DNA sequence at the junction of the pelB leader and the 5'-end of the BRIP gene was verified by DNA sequence analysis. This vector was denoted pING3321-1.

The final expression vector was assembled by placing the BRIP gene under the control of the inducible araB promoter. Plasmid pING3321-1 was cut with *Pst*I and

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XhoI, and the BRIP gene linked to the *pelB* leader was purified from an agarose gel. The expression vector pING3217, containing the *araB* promoter, was cut with *PstI* and *XhoI* and ligated to the BRIP gene. The expression vector was denoted pING3322.

Arabinose induction of *E. coli* cells containing the plasmid pING3322 in a fermenter resulted in the production of about 100 mg per liter of recombinant BRIP. *E. coli*-produced BRIP displays properties identical to BRIP purified directly from barley seeds.

D.   Construction Of BRIP Analogs
      With A Free Cysteine Residue

The BRIP protein contains no cysteine residues, and therefore contains no residues directly available which may form a disulfide linkage to antibodies or other proteins. Analogs of recombinant BRIP were generated which contain a free cysteine residue near the C-terminus of the protein. Three residues of the BRIP protein were targets for amino acid substitutions. Comparison of the amino acid sequence of BRIP to the known tertiary structure of the ricin A-chain (see FIG. 2) suggested that the three positions would be available near the surface of the molecule. The three BRIP analogs include cysteines substituted in place of serine$_{277}$, alanine$_{270}$, and leucine$_{256}$ of the native protein, and were designated BRIP$_{C277}$ (SEQ ID NO: 127), BRIP$_{C270}$ (SEQ ID NO: 128) and BRIP$_{C256}$ (SEQ ID NO: 129), respectively.

(1)   A plasmid vector capable of expressing the BRIP$_{C277}$ analog was constructed by replacing the 3'-end of the BRIP gene with a DNA segment conferring the amino acid change. The *EcoRI* fragment containing the BRIP gene from pBS1 was subcloned into M13mp18, and single-stranded DNA (anti-sense strand) was amplified by PCR with primers OBM2 (corresponding nucleotides -11 to +8 of the BRIP gene) and OMB4 (corresponding to amino acids 264-280 of BRIP and the termination codon of BRIP, and incorporating the

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substitution of a cysteine codon for the native codon for serine$_{277}$ of native BRIP). The sequences of primers OBM2 and OMB4, wherein the underlined nucleotides encode the substituted cysteine, are set out below.

5
                    OBM2   (SEQ ID NO: 47)
                    5' GCATTACATCCATGGCGGC 3'
                    OMB4 (SEQ ID NO: 48)
                    5' GATATCTCGAGTTAACTATTTCCCACC<u>ACA</u>CG
                    CATGGAACAGCTCCAGCGCCTTGGCCACCGTC 3'

10   A fragment containing a BRIP gene in which the codon for the amino acid at position 277 was changed to a cysteine codon was amplified. The fragment was cloned into the *Sma*I site of pUC19 (BRL) and the plasmid generated was denoted pMB22. pMB22 was digested with *Eco*RI and an *Eco*RI-*Xho*I
15   linker (Clonetech, Palo Alto, CA) was ligated into the vector. Subsequent digestion with *Xho*I and religation generated vector pINGMB2X. A *Bam*HI to *Xho*I fragment encoding the 3'-end of BRIP with the altered amino acid was excised from pMB2X and the fragment was purified on a 5%
20   acrylamide gel. This fragment along with an *Eco*RI to *Bam*HI fragment containing the *pel*B leader sequence and sequences encoding the first 256 amino acids of BRIP were substituted in a three piece ligation into pING3322 cut with *Eco*RI and *Xho*I. The resulting vector containing the BRIP$_{C277}$ analog
25   was designated pING3803 (ATCC Accession No. 68722).

            (2)   A BRIP analog with a free cysteine at position 256 was constructed using PCR to introduce the amino acid substitution. A portion of the expression plasmid pING3322 was amplified with primers BRIP-256 and
30   *HIND*III-2. The sequence of each primer is set out below.
                    BRIP-256 (SEQ ID NO: 49)
                    5' <u>TGT</u>CTGTTCGTGGAGGTGCCG 3'
                    *HIND*III-2 (SEQ ID NO: 44)
                    5' CGTTAGCAATTTAACTGTGAT 3'

35   Nucleotides 4-21 of primer BRIP-256 encode amino acids 256-262 of BRIP while the underlined nucleotides specify the cysteine to be substituted for the leucine at the

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corresponding position of the native BRIP protein. Primer *HINDIII-2* corresponds to a portion of the plasmid. The PCR product, which encodes the carboxyl terminal portion of the BRIP analog, was treated with T4 polymerase, cut with *XhoI*, and the resulting fragment was purified on a 5% acrylamide gel. Concurrently, plasmid pING3322 was cut with *BamHI*, treated with T4 polymerase, cut with *EcoRI*, and the fragment containing the *pelB* leader sequence and sequences encoding the first 256 amino acids of BRIP was purified. The two fragments were then assembled back into pING3322 to generate the gene encoding the analog $BRIP_{C256}$. This plasmid is denoted pING3801.

(3)   A BRIP analog with a cysteine at position 270 was also generated using PCR. A portion of the expression plasmid pING3322 was amplified with primers BRIP-270 and the *HINDIII-2* primer (SEQ ID NO: 44). The sequence of primer BRIP-270 is set out below.

BRIP-270 (SEQ ID NO: 50)

5' CCAAGTGTCTGGAGCTGTTCCATGCGA 3'

Primer BRIP-270 corresponds to amino acids 268-276 of BRIP with the exception of residue 270. The codon of the primer corresponding to position 270 specifies a cysteine instead of the alanine present in the corresponding position in native BRIP. The PCR product was treated with T4 polymerase, cut with *XhoI*, and the 51 bp fragment, which encodes the carboxyl terminal portion of the analog, was purified on a 5% acrylamide gel. The fragment (corresponding to amino acids 268-276 of $BRIP_{C270}$) was cloned in a three piece ligation along with the internal 151 bp BRIP restriction fragment from *SstII* to *MscI* (corresponding to BRIP amino acids 217-267) from plasmid pING3322, and restriction fragment from *SstII* to *XhoI* from pING3322 containing the remainder of the BRIP gene. The plasmid generated contains the gene encoding the $BRIP_{C270}$ analog and is designated pING3802.

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E.   Purification Of Recombinant
      BRIP And The BRIP Analogs

            Recombinant BRIP (rBRIP) and the BRIP analogs
with free cysteine residues were purified essentially as
described for native BRIP except they were prepared from
concentrated fermentation broths. For rBRIP, concentrated
broth from a 10 liter fermentation batch was exchanged into
10 mM Tris, 20 mM NaCl pH 7.5, loaded onto a Sephacryl S-
200 column, and eluted with a 20 to 500 mM NaCl linear
gradient.   Pooled rBRIP was further purified on a Blue
Toyopearl® column (TosoHaas) loaded in 20 mM NaCl and
eluted in a 20 to 500 mM NaCl gradient in 10mM Tris, pH
7.5.   For BRIP analogs, concentrated fermentation broths
were loaded onto a CM52 column (Whatman) in 10 mM phosphate
buffer, pH 7.5, and eluted with a 0 to 0.3M NaCl linear
gradient.   Further purification was by chromatography on a
Blue Toyopearl® column.

F.   Reticulocyte Lysate Assay

            The ability of the rBRIP and the BRIP analogs to
inhibit protein synthesis *in vitro* was tested by
reticulocyte lysate assay as described in Example 1.
Serial log dilutions of standard toxin (RTA 30), native
BRIP, rBRIP and BRIP analogs were tested over a range of 1
μg/ml to 1 pg/ml.   By comparison with an uninhibited
sample, the picomolar concentration of toxin (pM) which
corresponds to 50% inhibition of protein synthesis (IC_{50})
was calculated.   The results of the assays are presented
below in Table 17.

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Table 17

Toxin	IC$_{50}$ (pM)
RTA 30	3.1
Native BRIP	15
rBRIP	18
BRIP$_{C256}$	23
BRIP$_{C270}$	20
BRIP$_{C277}$	24

The RLA results indicate that the BRIP analogs exhibit ribosome-inactivating activity comparable to that of the recombinant and native BRIP toxin. All the analogs retained the natural ability of native BRIP to inhibit protein synthesis, suggesting that amino acid substitution at these positions does not affect protein folding and activity.

Example 23

Construction Of BRIP Immunoconjugates

Immunoconjugates of native BRIP (SEQ ID NO: 3) with 4A2 (described in Morishima *et al.*, *J. Immunol.*, *129*:1091 (1982) and H65 antibody (obtained from hybridoma ATCC HB9286) which recognize the T-cell determinants CD7 and CD5, respectively, were constructed. Immunoconjugates of ricin A-chains (RTAs) with 4A2 and H65 antibody were constructed as controls. The H65 antibody and ricin A-chains as well as the RTA immunoconjugates were prepared and purified according to methods described in U.S. Patent Application Serial No. 07/306,433 *supra* and in International Publication No. WO 89/06968.

To prepare immunoconjugates of native BRIP, both the antibody (4A2 or H65) and native BRIP were chemically modified with the hindered linker 5-methyl-2-iminothiolane (M2IT) at lysine residues to introduce a reactive sulfhydryl group as described in Goff *et al.*, *Bioconjugate Chem.*, *1*:381-386 (1990). BRIP (3 mg/ml) was first incubated with 0.5 mM M2IT and 1 mM DTNB in 25 mM

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triethanolamine, 150 mM NaCl, pH 8.0, for 3 hours at 25°C. The derivitized BRIP-(M2IT)-S-S-TNB was then desalted on a column of Sephadex GF-05LS and the number of thiol groups introduced was quantitated by the addition of 0.1 mM DTT. On average, each BRIP molecule contained 0.7 SH/mol.

4A2 or H65 antibody (4 mg/ml) in triethanolamine buffer was similarly incubated with M2IT (0.3 mM) and DTNB (1 mM) for 3 hours at 25°C. Antibody-(M2IT)-S-S-TNB was then desalted and the TNB:antibody ratio was determined. To prepare the conjugate, the BRIP-(M2IT)-S-S-TNB was first reduced to BRIP-(M2IT)-SH by treatment with 0.5 mM DTT for 1 hour at 25°C, desalted by gel filtration of Sephadex® GF-05LS to remove the reducing agent, and then mixed with antibody-(M2IT)-S-S-TNB.

Following a 3 hour incubation at 25°C, and an additional 18 hours at 4°C, the conjugate was purified by sequential chromatography on AcA44 (IBF) and Blue Toyopearl®. Samples of the final product were run on 5% non-reducing SDS PAGE, Coomassie stained, and scanned with a Shimadzu laser densitometer to quantitate the number of toxins per antibody.

The BRIP analogs containing a free cysteine were also conjugated to 4A2 and H65 antibodies. The analogs were treated with 50 mM DTT either for 2 hours at 25°C or for 18 hours at 4°C to expose the reactive sulfhydryl group of the cysteine and desalted. The presence of a free sulfhydryl was verified by reaction with DTNB [Ellman et al., Arch. Biochem. Biophys, 82:70-77 (1959)]. 4A2 or H65 antibody derivatized as described above with M2IT was incubated with the reduced BRIP analogs at a ratio of 1:5 at room temperature for 3 hours and then overnight at 4°C. Immunoconjugates H65-BRIP$_{C256}$, 4A2-BRIP$_{C256}$, H65-BRIP$_{C277}$ were prepared in 25 mM triethanolamine, 150 mM NaCl pH 8, while immunoconjugates H65-BRIP$_{C270}$, 4A2-BRIP$_{C270}$ and 4A2-BRIP$_{C277}$ were prepared in 0.1 M sodium phosphate, 150 mM NaCl pH 7.5. Following conjugation, 10 μM mercaptoethylamine was added for 15 minutes at 25°C to quenched any unreacted m2IT

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linkers on the antibody. The quenched reaction solution
was promptly loaded onto a gel filtration column (AcA44) to
remove uncojugated ribosome-inactivating protein.
Purification was completed using soft gel affinity
chromatography on Blue Toyopearl® resin using a method
similar to Knowles *et al.*, *Analyt. Biochem.*, *160*:440
(1987). Samples of the final product were run on 5% non-
reduced SDS PAGE, Coomassie stained, and scanned with a
Shimadzu laser densitometer to quantitate the number of
toxins per antibody. The conjugation efficiency was
substantially greater for $BRIP_{C277}$ (78%) than for either of
the other two analogs, $BRIP_{C270}$ and $BRIP_{C266}$ (each of these was
about 10%). Additionally, the $BRIP_{C277}$ product was a
polyconjugate, *i.e.*, several BRIP molecules conjugated to
a single antibody, in contrast to the $BRIP_{C270}$ and $BRIP_{C256}$
products which were monoconjugates.

Example 24

Properties Of BRIP Immunoconjugates

A.   Whole Cell Kill Assay

Immunoconjugates of native BRIP and of the BRIP
analogs were tested for the ability to inhibit protein
synthesis in HSB2 cells by the whole cell kill assay
described in Example 1. Standard immunoconjugates H65-RTA
(H65 derivatized with SPDP linked to RTA) and 4MRTA (4A2
antibody derivatized with M2IT linked to RTA) and BRIP
immunoconjugate samples were diluted with RPMI without
leucine at half-log concentrations ranging from 2000 to
0.632 ng/ml. All dilutions were added in triplicate to
microtiter plates containing 1×10^5 HSB2 cells. HSB2
plates were incubated for 20 hours at 37°C and then pulsed
with ^{3}H-Leu for 4 hours before harvesting. Samples were
counted on the Inotec Trace 96 cascade ionization counter.
By comparison with an untreated sample, the picomolar toxin
concentration (pM T) of immunoconjugate which resulted in
a 50% inhibition of protein synthesis (IC_{50}) was calculated.
The assay results are presented below in Table 18.

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Table 18

Conjugate	IC_{50} (pM T)
4A2-BRIP	122.45
4A2-BRIP$_{C270}$	46.3
4A2-BRIP$_{C277}$	57.5
4A2-BRIP$_{C256}$	1116
H65-BRIP	>5000
H65-BRIP$_{C277}$	1176

The BRIP analog conjugates were less potent than the ricin conjugate control (data not shown). The immunotoxins containing antibody 4A2 and either the BRIP$_{C270}$ or the BRIP$_{C277}$ analog exhibited comparable to increased specific cytotoxicity toward target cells as compared to immunotoxin containing native BRIP. While 4A2-BRIP$_{C256}$ is less active than 4A2-BRIP, 4A2-BRIP$_{C270}$ and 4A2-BRIP$_{C277}$ are between 3 and 4 times more active. Similarly, the immunoconjugate of H65 to BRIP$_{C277}$ shows greater toxicity toward target cells than the immunoconjugate of H65 to native BRIP. Thus, linkage of antibody to BRIP derivatives which have an available cysteine residue in an appropriate location results in immunotoxins with enhanced specific toxicity toward target cells relative to conjugates with native BRIP.

B.   Disulfide Bond Stability Assay

Immunoconjugates prepared with native BRIP and the BRIP analogs were examined by the disulfide bond stability assay described in Example 1. Briefly, conjugates were incubated with increasing concentrations of glutathione for 1 hour at 37°C and, after terminating the reaction with iodoacetamide, the amount of RIP released was quantitated by size-exclusion HPLC on a TosoHaas TSK-G2000SW column.

By comparisons with the amount of RIP released by high concentrations of 2-mercaptoethanol (to determine 100% release), the concentration of glutathione required to

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release 50% of the RIP (the RC_{50}) was calculated. As shown below in Table 19, the conjugates prepared with $BRIP_{C270}$ or $BRIP_{C277}$ were significantly more stable than either the RTA conjugates or those prepared with native BRIP.

5

Table 19

Conjugate	RC_{50} (mM)
H65-RTA	7.0
H65-BRIP	2.8
H65-BRIPC277	196.0
4A2-RTA	4.4
4A2-BRIP	3.3
42-$BRIP_{C270}$	53.0
4A2-$BRIP_{C277}$	187.0

These unexpected results suggest that conjugates prepared with Type I RIP analogs according to the present invention may have enhanced stability and efficacy *in vivo*.

Example 25

Preparation of Momordin and Analogs Thereof

Plants of the genus *Momordica* produce a number of related proteins known as momordins or momorcharins which are Type I RIPs. The gene encoding momordin II was cloned from *Momordica balsamina* seeds.

A.   Preparation Of *M. balsamina* RNA

Total RNA was prepared from 4 g of *M. balsamina* seeds as described in Ausubel et *al., supra*.   PolyA containing RNA was prepared from 1 mg of total RNA by chromatography on oligo-(dT)-cellulose.   40 mg of oligo-(dT)-cellulose Type 7 (Pharmacia) was added to 0.1 N NaOH and poured into a disposable column (Biorad).   The column was washed with water until the eluate was pH 5.5, and then was washed with 1X loading buffer (50 mM NaCitrate, 0.5M NaCl, 1 mM EDTA, 0.1% SDS, pH 7.0) until the eluate was pH 7.0.   1 mg of total RNA was suspended in 300 μl of water,

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heated to 65°C for 5 minutes, and 300 µl of 2X loading
buffer was added (100 mM Na Citrate, 1M NaCl, 2 mM EDTA,
and 0.2% SDS). The RNA was loaded onto the column, and the
flow through was reheated to 65°C, cooled to room
5   temperature, and reloaded onto the column. Column-bound
mRNA was washed 5 times with 0.5 ml of 1X loading buffer,
and two times with 0.5 ml of 0.05M NaCitrate, 0.1 M NaCl,
1 mM EDTA, 0.1% SDS. PolyA- containing RNA was eluted two
times from the column with 0.5 ml of 25 mM NaCitrate, 1 mM
10 : EDTA, and 0.05% SDS.

B.   Library Preparation

        A cDNA library from the polyA-containing *M.*
balsamina RNA was prepared in a bacterial expression
plasmid with the SuperScript Plasmid System (BRL,
15   Gaithersburg, Maryland). The cDNA was synthesized from 2
µg of poly A-containing RNA, size fractionated, digested
with *NotI*, and ligated into the expression vector pSPORT as
recommended by the manufacturer of the vector, BRL.

C.   Cloning Of The Momordin II Gene

20   A DNA fragment encoding the first 27 amino acids
of momordin II was amplified from *M. balsamina* cDNA by PCR.
First strand cDNA was prepared from 100 ng of polyA
containing RNA with an RNA-PCR Kit (Perkin Elmer Cetus).
Two partially degenerate primers were synthesized based on
25   the amino acid sequence of the first 27 amino acids of
momordin II described in Li *et al.*, *Experientia*, *36*:524-527
(1980). Because the amino acid sequence of amino acids 1-
27 of momordin II is 52% homologous to amino acids 1-17 of
momordin I [Ho *et al.*, *BBA,* *1088*:311-314 (1991)], some
30   codon assignments in the degenerate primers were based on
homology to the corresponding amino acid as well as codon
preference in the momordin I gene. The sequences of
primers momo-3 and momo-4 are set out below using IUPAC
nucleotide symbols.

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momo-3 (SEQ ID NO: 51)

5' GATGTTAAYTTYGAYTTGTCNACDGCTAC 3'

momo-4 (SEQ ID NO: 52)

5' ATTGGNAGDGTAGCCCTRAARTCYTCDAT 3'

5   The resulting 81 bp PCR product was purified on a 5%
acrylamide gel and cloned into the *Sma*I site of pUC18.
Three candidate clones were sequenced, and one clone,
pMO110, was identified which encoded the N-terminal 27
amino acids of momordin II.

10   A hybridization probe was designed for screening
of the momordin II cDNA library based on the sequence of
the pMO110 momordin II DNA fragment. The sequence of the
primer momo-5 is shown below.

momo-5 (SEQ ID NO: 53)

15   5' GCCAC<u>TGCAAAAACCTACACAAAATTT</u>ATTGA 3'
Primer momo-5 corresponds to amino acids 9-18 of mature
momordin II. The underlined nucleotides of the primer were
expected to match the DNA sequence of the momordin II gene
exactly. Since this sequence is highly A/T-rich and may

20   hybridize to the momordin II gene weakly, the additional
adjacent nucleotides were included in the primer. Bases 3
and 30 (overlined) were in the "wobble" position (*i.e.*; the
third nucleotide in a codon) of amino acids 9 (alanine) and
18 (isoleucine), respectively, of momordin II and may not

25   be identical to the nucleotide bases in the native gene.

A 90,000 member cDNA library in pSPORT was
screened with ^{32}P-kinased momo-5, and eight potential
candidate clones were identified. One clone, pING3619, was
sequenced and contains an open reading frame corresponding

30   in part to the expected N-terminal 27 residues of Momordin
II. The complete momordin gene contains 286 amino acids,
the first 23 of which are a presumed leader signal (mature
momordin II is 263 residues). The DNA sequence of the
momordin II gene is set out in SEQ ID NO: 13.

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D.   Construction Of An Expression
      Vector Containing The Momordin II Gene

            A bacterial expression vector for the momordin II
gene was constructed.   Two PCR primers were synthesized,
one (momo-9) which primes from the +1 residue of the mature
momordin II amino acid sequence, and one at the C-terminus
(momo-10) of momordin II which introduces an *XhoI*
restriction site:

               momo-9 (SEQ ID NO: 54)
                  5' GATGTTAACTTCGATTTGTCGA 3'
               momo-10 (SEQ ID NO: 55)
                  5' TCAACTCGAGGTACTCAATTCACAACAGATTCC 3'

pING3619 was amplified with momo-9 and momo-10, and the
product was treated with T4 polymerase, cut with *XhoI*, and
purified on an agarose gel.   This gene fragment was ligated
along with the 131 bp *pelB* leader fragment from pIC100
which has been generated by *SstI* digestion, T4-polymerase
treatment, and *EcoRI* digestion, into the araB expression
vector cleaved with *EcoRI* and *XhoI*.   The product of this
three piece ligation was sequenced to verify that the *pelB*
junction and momordin II coding sequence were correct.
Arabinose induction of cells containing the momordin II
expression plasmid pING3621 results in production of
momordin II in *E. coli*.

E.   Analogs Of Mormordin II

            Mormordin II has no natural cysteines available
for conjugation to antibody.   Analogs of momordin which
have a free cysteine for conjugation to an antibody may be
constructed.   Positions likely to be appropriate for
substitution of a cysteine residue may be identified from
Figure 3 as positions near the ricin A-chain $cysteine_{259}$ and
as positions including the last 26 amino acids of momordin
II that are accessible to solvent.   For example, the
arginine at position 242 of momordin II aligns with the
ricin A-chain cysteine at position 259 and is a preferred
target for substitution.   Additional preferred substitution

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positions for momordin II include the serine at position 241 and the alanine at position 243.

      While the present invention has been described in terms of preferred embodiments, it is understood that variations and improvements will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: Xoma Corporation

    (ii) TITLE OF INVENTION: Immunotoxins Comprising Ribsome-Inactivating Proteins

    (iii) NUMBER OF SEQUENCES: 144

    (iv) CORRESPONDENCE ADDRESS:
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        (F) ZIP: 60606-6402

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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        (B) FILING DATE:

    (vii) PRIOR APPLICATION DATA:
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        (B) FILING DATE: 12-MAY-1993

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        (A) APPLICATION NUMBER: US 07/988,430
        (B) FILING DATE: 09-DEC-1992

    (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: PCT US92/09487
        (B) FILING DATE: 04-NOV-1992

    (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 07/901,707
        (B) FILING DATE: 19-JUN-1992

    (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: US 07/787,567
        (B) FILING DATE: 04-NOV-1991

    (viii) ATTORNEY/AGENT INFORMATION:
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        (C) REFERENCE/DOCKET NUMBER: 32088

    (ix) TELECOMMUNICATION INFORMATION:
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        (B) TELEFAX: 312/474-0448
        (C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 267 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr Ala Gly Ala
1   5   10   15

Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg Gly Arg Leu
        20   25   30

Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu Pro Asn Arg
        35   40   45

Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu Leu Ser Asn
    50   55   60

His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr Asn Ala Tyr
65   70   75   80

Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe His Pro Asp
            85   90   95

Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr Asp Val Gln
        100   105   110

Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg Leu Glu Gln
        115   120   125

Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn Gly Pro Leu
    130   135   140

Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly Gly Thr Gln
145   150   155   160

Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln Met Ile Ser
            165   170   175

Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg Thr Arg Ile
        180   185   190

Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile Thr Leu Glu
        195   200   205

Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser Asn Gln Gly
        210   215   220

Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly Ser Lys Phe
225   230   235   240

Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala Leu Met Val
            245   250   255

Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe
            260   265

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5                   10                  15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20                  25                  30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35                  40                  45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50                  55                  60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70                  75                  80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85                  90                  95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100                 105                 110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115                 120                 125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130                 135                 140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145                 150                 155                 160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165                 170                 175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180                 185                 190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195                 200                 205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210                 215                 220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225                 230                 235                 240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 280 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Ala Ala Lys Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala Thr
1               5                   10                  15
```

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```
Phe Asn Val Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala Gly
            20              25                 30

Ile Arg Asn Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg Pro
        35                  40                 45

Val Leu Pro Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe His
    50              55              60

Val Val Leu Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala Ile
65              70              75                      80

Arg Ala Asp Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly Thr
            85              90                  95

Trp Trp Glu Leu Thr Pro Gly Leu Ile Pro Gly Ala Thr Tyr Val Gly
            100             105             110

Phe Gly Gly Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu Thr
        115             120             125

Asn Val Ala Leu Gly Arg Gln Gln Leu Ala Asp Ala Val Thr Ala Leu
    130             135             140

His Gly Arg Thr Lys Ala Asp Lys Ala Ser Gly Pro Lys Gln Gln Gln
145             150             155             160

Ala Arg Glu Ala Val Thr Thr Leu Val Leu Met Val Asn Glu Ala Thr
            165             170             175

Arg Phe Gln Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro Lys
        180             185             190

Ala Val Glu Lys Lys Ser Gly Lys Ile Gly Asn Glu Met Lys Ala Gln
        195             200             205

Val Asn Gly Trp Gln Asp Leu Ser Ala Ala Leu Leu Lys Thr Asp Val
    210             215             220

Lys Pro Pro Pro Gly Lys Ser Pro Ala Lys Phe Ala Pro Ile Glu Lys
225             230             235             240

Met Gly Val Arg Thr Ala Glu Gln Ala Ala Asn Thr Leu Gly Ile Leu
            245             250             255

Leu Phe Val Glu Val Pro Gly Gly Leu Thr Val Ala Lys Ala Leu Glu
            260             265             270

Leu Phe His Ala Ser Gly Gly Lys
            275             280
```

(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 263 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Asp Val Asn Phe Asp Leu Ser Thr Ala Thr Ala Lys Thr Tyr Thr Lys
1               5               10                  15
```

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```
Phe Ile Glu Asp Phe Arg Ala Thr Leu Pro Phe Ser His Lys Val Tyr
        20              25                  30

Asp-Ile-Pro-Leu-Leu-Tyr-Ser-Thr-Ile-Ser-Asp-Ser-Arg Arg Phe Ile
        35              40                  45

Leu Leu Asp Leu Thr Ser Tyr Ala Tyr Glu Thr Ile Ser Val Ala Ile
    50              55                  60

Asp Val Thr Asn Val Tyr Val Val Ala Tyr Arg Thr Arg Asp Val Ser
65              70              75                  80

Tyr Phe Phe Lys Glu Ser Pro Pro Glu Ala Tyr Asn Ile Leu Phe Lys
            85              90                  95

Gly Thr Arg Lys Ile Thr Leu Pro Tyr Thr Gly Asn Tyr Glu Asn Leu
            100             105                 110

Gln Thr Ala Ala His Lys Ile Arg Glu Asn Ile Asp Leu Gly Leu Pro
        115             120                 125

Ala Leu Ser Ser Ala Ile Thr Thr Leu Phe Tyr Tyr Asn Ala Gln Ser
    130             135                 140

Ala Pro Ser Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu Ala Ala
145             150                 155                 160

Arg Phe Lys Tyr Ile Glu Arg His Val Ala Lys Tyr Val Ala Thr Asn
            165                 170                 175

Phe Lys Pro Asn Leu Ala Ile Ile Ser Leu Glu Asn Gln Trp Ser Ala
            180                 185                 190

Leu Ser Lys Gln Ile Phe Leu Ala Gln Asn Gln Gly Gly Lys Phe Arg
        195             200             205

Asn Pro Val Asp Leu Ile Lys Pro Thr Gly Glu Arg Phe Gln Val Thr
    210             215                 220

Asn Val Asp Ser Asp Val Val Lys Gly Asn Ile Lys Leu Leu Leu Asn
225             230                 235                 240

Ser Arg Ala Ser Thr Ala Asp Glu Asn Phe Ile Thr Thr Met Thr Leu
            245             250                 255

Leu Gly Glu Ser Val Val Asn
            260
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 248 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Asp Val Arg Phe Ser Leu Ser Gly Ser Ser Ser Thr Ser Tyr Ser Lys
1               5                   10                  15

Phe Ile Gly Asp Leu Arg Lys Ala Leu Pro Ser Asn Gly Thr Val Tyr
        20              25                  30
```

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```
Asn Leu Thr Ile Leu Leu Ser Ser Ala Ser Gly Ala Ser Arg Tyr Thr
        35              40              45

Leu Met Thr Leu Ser Asn Tyr Asp Gly Lys Ala Ile Thr Val Ala Val
        50              55              60

Asp Val Ser Gln Leu Tyr Ile Met Gly Tyr Leu Val Asn Ser Thr Ser
65              70              75              80

Tyr Phe Phe Asn Glu Ser Asp Ala Lys Leu Ala Ser Gln Tyr Val Phe
            85              90              95

Lys Gly Ser Thr Ile Val Thr Leu Pro Tyr Ser Gly Asn Tyr Glu Lys
            100             105             110

Leu Gln Thr Ala Ala Gly Lys Ile Arg Glu Lys Ile Pro Leu Gly Phe
            115             120             125

Pro Ala Leu Asp Ser Ala Leu Thr Thr Ile Phe His Tyr Asp Ser Thr
    130             135             140

Ala Ala Ala Ala Ala Phe Leu Val Ile Leu Gln Thr Thr Ala Glu Ala
145             150             155             160

Ser Arg Phe Lys Tyr Ile Glu Gly Gln Ile Ile Glu Arg Ile Ser Lys
            165             170             175

Asn Gln Val Pro Ser Leu Ala Thr Ile Ser Leu Glu Asn Ser Leu Trp
            180             185             190

Ser Ala Leu Ser Lys Gln Ile Gln Leu Ala Gln Thr Asn Asn Gly Thr
        195             200             205

Phe Lys Thr Pro Val Val Ile Thr Asp Asp Lys Gly Gln Arg Val Glu
    210             215             220

Ile Thr Asn Val Thr Ser Lys Val Val Thr Lys Asn Ile Gln Leu Leu
225             230             235             240

Leu Asn Tyr Lys Gln Asn Val Ala
            245
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 255 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Asp Val Ser Phe Arg Leu Ser Gly Ala Thr Ser Ser Ser Tyr Gly Val
1           5               10              15

Phe Ile Ser Asn Leu Arg Lys Ala Leu Pro Asn Glu Arg Lys Leu Tyr
            20              25              30

Asp Leu Pro Leu Ile Arg Ser Ser Leu Pro Gly Ser Gln Arg Tyr Ala
        35              40              45

Ile Ile His Leu Thr Asn Tyr Ala Asp Glu Val Ala Leu Asp Val Thr
    50              55              60
```

```
Asn Val Asp Ala Gly Leu Pro Arg Asn Ala Val Leu Tyr Ile Met Gly
65              70              75              80

Tyr Arg Ala Gly Asp Thr Ser Tyr Phe Phe Asn Glu Ala Ser Ala Thr
                85              90              95

Glu Ala Ala Lys Tyr Val Phe Lys Asp Ala Met Arg Lys Val Thr Leu
            100             105             110

Pro Tyr Ser Gly Asn Tyr Glu Arg Leu Gln Thr Ala Ala Gly Gly Leu
        115             120             125

Arg Glu Asn Ile Pro Leu Gly Leu Pro Ala Leu Asp Ser Ala Ile Thr
    130             135             140

Thr Leu Phe Tyr Tyr Asn Ala Asn Ser Ala Ala Ser Ala Leu Met Val
145             150             155             160

Leu Ile Gln Ser Thr Ser Glu Ala Ala Arg Tyr Lys Phe Ile Glu Gln
                165             170             175

Gln Ile Gly Ser Arg Val Asp Lys Thr Phe Leu Pro Ser Leu Ala Ile
            180             185             190

Ile Ser Leu Glu Asn Ser Leu Trp Leu Ala Leu Ser Lys Gln Ile Gln
        195             200             205

Ile Ala Ser Thr Asn Asn Gly Glu Phe Glu Thr Pro Val Val Leu Ile
    210             215             220

Asn Ala Gln Asn Gln Arg Val Thr Ile Thr Asn Val Asp Ala Gly Val
225             230             235             240

Val Thr Ser Asn Ile Ala Leu Leu Leu Asn Arg Asn Asn Met Ala
            245             250             255
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 263 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Asp Val Ser Phe Arg Leu Ser Gly Ala Asp Pro Arg Ser Tyr Gly Met
1           5               10              15

Phe Ile Lys Asp Leu Arg Asn Ala Leu Pro Phe Arg Glu Lys Val Tyr
            20              25              30

Asn Ile Pro Leu Leu Leu Pro Ser Val Ser Gly Ala Gly Arg Tyr Leu
        35              40              45

Leu Met His Leu Phe Asn Tyr Asp Gly Lys Thr Ile Thr Val Ala Val
        50              55              60

Asp Val Thr Asn Val Tyr Ile Met Gly Tyr Leu Ala Asp Thr Thr Ser
65              70              75              80

Tyr Phe Phe Asn Glu Pro Ala Ala Glu Leu Ala Ser Gln Tyr Val Phe
            85              90              95
```

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```
Arg Asp Ala Arg Arg Lys Ile Thr Leu Pro Tyr Ser Gly Asn Tyr Glu
            100             105         110

Arg Leu Gln Ile Ala Ala Gly Lys Pro Arg Glu Lys Ile Pro Ile Gly
            115             120         125

Leu Pro Ala Leu Asp Ser Ala Ile Ser Thr Leu Leu His Tyr Asp Ser
    130             135             140

Thr Ala Ala Ala Gly Ala Leu Leu Val Leu Ile Gln Thr Thr Ala Glu
145             150             155             160

Ala Ala Arg Phe Lys Tyr Ile Glu Gln Gln Ile Gln Glu Arg Ala Tyr
            165             170             175

Arg Asp Glu Val Pro Ser Leu Ala Thr Ile Ser Leu Glu Asn Ser Trp
            180             185             190

Ser Gly Leu Ser Lys Gln Ile Gln Leu Ala Gln Gly Asn Asn Gly Ile
        195             200             205

Phe Arg Thr Pro Ile Val Leu Val Asp Asn Lys Gly Asn Arg Val Gln
    210             215             220

Ile Thr Asn Val Thr Ser Lys Val Val Thr Ser Asn Ile Gln Leu Leu
225             230             235             240

Leu Asn Thr Arg Asn Ile Ala Glu Gly Asp Asn Gly Asp Val Ser Thr
            245             250             255

Thr His Gly Phe Ser Ser Thr
            260
```

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 250 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Ala Pro Thr Leu Glu Thr Ile Ala Ser Leu Asp Leu Asn Asn Pro Thr
1               5               10              15

Thr Tyr Leu Ser Phe Ile Thr Asn Ile Arg Thr Lys Val Ala Asp Lys
            20              25              30

Thr Glu Gln Cys Thr Ile Gln Lys Ile Ser Lys Thr Phe Thr Gln Arg
        35              40              45

Tyr Ser Tyr Ile Asp Leu Ile Val Ser Ser Thr Gln Lys Ile Thr Leu
    50              55              60

Ala Ile Asp Met Ala Asp Leu Tyr Val Leu Gly Tyr Ser Asp Ile Ala
65              70              75              80

Asn Asn Lys Gly Arg Ala Phe Phe Phe Lys Asp Val Thr Glu Ala Val
            85              90              95

Ala Asn Asn Phe Phe Pro Gly Ala Thr Gly Thr Asn Arg Ile Lys Leu
            100             105             110
```

```
Thr Phe Thr Gly Ser Tyr Gly Asp Leu Glu Lys Asn Gly Gly Leu Arg
        115             120             125

Lys Asp Asn Pro Leu Gly Ile Phe Arg Leu Glu Asn Ser Ile Val Asn
        130             135             140

Ile Tyr Gly Lys Ala Gly Asp Val Lys Lys Gln Ala Lys Phe Phe Leu
145             150             155             160

Leu Ala Ile Gln Met Val Ser Glu Ala Ala Arg Phe Lys Tyr Ile Ser
                165             170             175

Asp Lys Ile Pro Ser Glu Lys Tyr Glu Glu Val Thr Val Asp Glu Tyr
            180             185             190

Met Thr Ala Leu Glu Asn Asn Trp Ala Lys Leu Ser Thr Ala Val Tyr
        195             200             205

Asn Ser Lys Pro Ser Thr Thr Thr Ala Thr Lys Cys Gln Leu Ala Thr
        210             215             220

Ser Pro Val Thr Ile Ser Pro Trp Ile Phe Lys Thr Val Glu Glu Ile
225             230             235             240

Lys Leu Val Met Gly Leu Leu Lys Ser Ser
                245             250
```

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 261 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ile Asn Thr Ile Thr Phe Asp Ala Gly Asn Ala Thr Ile Asn Lys Tyr
1               5               10              15

Ala Thr Phe Met Glu Ser Leu Arg Asn Glu Ala Lys Asp Pro Ser Leu
        20              25              30

Lys Cys Tyr Gly Ile Pro Met Leu Pro Asn Thr Asn Ser Thr Ile Lys
        35              40              45

Tyr Leu Leu Val Lys Leu Gln Gly Ala Ser Leu Lys Thr Ile Thr Leu
    50              55              60

Met Leu Arg Arg Asn Asn Leu Tyr Val Met Gly Tyr Ser Asp Pro Tyr
65              70              75              80

Asp Asn Lys Cys Arg Tyr His Ile Phe Asn Asp Ile Lys Gly Thr Glu
            85              90              95

Tyr Ser Asp Val Glu Asn Thr Leu Cys Pro Ser Ser Asn Pro Arg Val
            100             105             110

Ala Lys Pro Ile Asn Tyr Asn Gly Leu Tyr Pro Thr Leu Glu Lys Lys
        115             120             125

Ala Gly Val Thr Ser Arg Asn Glu Val Gln Leu Gly Ile Gln Ile Leu
        130             135             140
```

```
        Ser Ser Lys Ile Gly Lys Ile Ser Gly Gln Gly Ser Phe Thr Glu Lys
        145             150             155             160

        Ile Glu Ala Asp Phe Leu Leu Val Ala Ile Gln Met Val Ser Glu Ala
                        165                 170             175

        Ala Arg Phe Lys Tyr Ile Glu Asn Gln Val Lys Thr Asn Phe Asn Arg
                        180             185             190

        Asp Phe Ser Pro Asn Asp Lys Val Leu Asp Leu Glu Glu Asn Trp Gly
                    195             200             205

        Lys Ile Ser Thr Ala Ile His Asn Ser Lys Asn Gly Ala Leu Pro Lys
            210             215                 220

        Pro Leu Glu Leu Lys Asn Ala Asp Gly Thr Lys Trp Ile Val Leu Arg
        225             230                 235             240

        Val Asp Glu Ile Lys Pro Asp Val Gly Leu Leu Asn Tyr Val Asn Gly
                        245             250             255

        Thr Cys Gln Ala Thr
                260
```

(2) INFORMATION FOR SEQ ID NO:10:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 259 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
        Val Thr Ser Ile Thr Leu Asp Leu Val Asn Pro Thr Ala Gly Gln Tyr
        1               5               10              15

        Ser Ser Phe Val Asp Lys Ile Arg Asn Asn Val Lys Asp Pro Asn Leu
                    20              25              30

        Lys Tyr Gly Gly Thr Asp Ile Ala Val Ile Gly Pro Pro Ser Lys Glu
                35              40              45

        Lys Phe Leu Arg Ile Asn Phe Gln Ser Ser Arg Gly Thr Val Ser Leu
            50              55              60

        Gly Leu Lys Arg Asp Asn Leu Tyr Val Val Ala Tyr Leu Ala Met Asp
        65              70              75              80

        Asn Thr Asn Val Asn Arg Ala Tyr Tyr Phe Arg Ser Glu Ile Thr Ser
                        85              90              95

        Ala Glu Ser Thr Ala Leu Phe Pro Glu Ala Thr Thr Ala Asn Gln Lys
                    100             105             110

        Ala Leu Glu Tyr Thr Glu Asp Tyr Gln Ser Ile Glu Lys Asn Ala Gln
                115             120             125

        Ile Thr Gln Gly Asp Gln Ser Arg Lys Glu Leu Gly Leu Gly Ile Asp
            130             135             140

        Leu Leu Ser Thr Ser Met Glu Ala Val Asn Lys Lys Ala Arg Val Val
        145             150             155             160
```

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```
        Lys Asp Glu Ala Arg Phe Leu Leu Ile Ala Ile Gln Met Thr Ala Glu
                    165                 170                 175

        Ala Ala Arg Phe Arg Tyr Ile Gln Asn Leu Val Ile Lys Asn Phe Pro
                    180                 185                 190

        Asn Lys Phe Asn Ser Glu Asn Lys Val Ile Gln Phe Glu Val Asn Trp
                    195                 200                 205

        Lys Lys Ile Ser Thr Ala Ile Tyr Gly Asp Ala Lys Asn Gly Val Phe
                    210                 215                 220

        Asn Lys Asp Tyr Asp Phe Gly Phe Gly Lys Val Arg Gln Val Lys Asp
        225                 230                 235                 240

        Leu Gln Met Gly Leu Leu Met Tyr Leu Gly Lys Pro Lys Ser Ser Asn
                    245                 250                 255

        Glu Ala Asn
```

(2) INFORMATION FOR SEQ ID NO:11:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 813 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GGGCTAGATA CCGTGTCATT CTCAACCAAA GGTGCCACTT ATATTACCTA CGTGAATTTC      60

TTGAATGAGC TACGAGTTAA ATTGAAACCC GAAGGTAACA GCCATGGAAT CCCATTGCTG     120

CGCAAAAAAT GTGATGATCC TGGAAAGTGT TTCGTTTTGG TAGCGCTTTC AAATGACAAT     180

GGACAGTTGG CGGAAATAGC TATAGATGTT ACAAGTGTTT ATGTGGTGGG CTATCAAGTA     240

AGAAACAGAT CTTACTTCTT TAAAGATGCT CCAGATGCTG CTTACGAAGG CCTCTTCAAA     300

AACACAATTA AAACAAGACT TCATTTTGGC GGCAGCTATC CCTCGCTGGA AGGTGAGAAG     360

GCATATAGAG AGACAACAGA CTTGGGCATT GAACCATTAA GGATTGGCAT CAAGAAACTT     420

GATGAAAATG CGATAGACAA TTATAAACCA ACGGAGATAG CTAGTTCTCT ATTGGTTGTT     480

ATTCAAATGG TGTCTGAAGC AGCTCGATTC ACCTTTATTG AGAACCAAAT TAGAAATAAC     540

TTTCAACAGA GAATTCGCCC GGCGAATAAT ACAATCAGCC TTGAGAATAA ATGGGGTAAA     600

CTCTCGTTCC AGATCCGGAC ATCAGGTGCA AATGGAATGT TTTCGGAGGC AGTTGAATTG     660

GAACGTGCAA ATGGCAAAAA ATACTATGTC ACCGCAGTTG ATCAAGTAAA ACCCAAAATA     720

GCACTCTTGA AGTTCGTCGA TAAAGATCCT AAAACGAGCC TTGCTGCTGA ATTGATAATC     760

CAGAACTATG AGTCATTAGT GGGCTTTGAT TAG                                 813
```

(2) INFORMATION FOR SEQ ID NO:12:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 846 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
ATGGCGGCAA AGATGGCGAA GAACGTGGAC AAGCCGCTCT TCACCGCGAC GTTCAACGTC      60

CAGGCCAGCT CCGCCGACTA CGCCACCTTC ATCGCCGGCA TCCGCAACAA GCTCCGCAAC     120

CCGGCGCACT TCTCCCACAA CCGCCCCGTG CTGCCGCCGG TCGAGCCCAA CGTCCCGCCG     180

AGCAGGTGGT TCCACGTCGT GCTCAAGGCC TCGCCGACCA GCGCCGGGCT CACGCTGGCC     240

ATCCGCGCGG ACAACATCTA CCTGGAGGGC TTCAAGAGCA GCGACGGCAC CTGGTGGGAG     300

CTCACCCCGG GCCTCATCCC CGGCGCCACC TACGTCGGGT TCGGCGGCAC CTACCGCGAC     360

CTCCTCGGCG ACACCGACAA GCTAACCAAC GTCGCTCTCG GCCGACAGCA GCTGGCGGAC     420

GCGGTGACCG CGCTCCACGG GCGCACCAAG GCCGACAAGG CCTCCGGCCC GAAGCAGCAG     480

CAGGCGAGGG AGGCGGTGAC GACGCTGGTC CTCATGGTGA ACGAGGCCAC GCGGTTCCAG     540

ACGGTGTCTG GGTTCGTGGC CGGGTTGCTG CACCCCAAGG CGGTGGAGAA GAAGAGCGGG     600

AAGATCGGCA ATGAGATGAA GGCCCAGGTG AACGGGTGGC AGGACCTGTC CGCGGCGCTG     660

CTGAAGACGG ACGTGAAGCC TCCGCCGGGA AAGTCGCCAG CGAAGTTCGC GCCGATCGAG     720

AAGATGGGCG TGAGGACGGC TGAACAGGCC GCCAACACGC TGGGGATCCT GCTGTTCGTG     780

GAGGTGCCGG GTGGGTTGAC GGTGGCCAAG GCGCTGGAGC TGTTCCATGC GAGTGGTGGG     840

AAATAG                                                                846
```

(2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 913 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CGTCCGAAAA TGGTGAAATG CTTACTACTT TCTTTTTTAA TTATCGCCAT CTTCATTGGT      60

GTTCCTACTG CCAAAGGCGA TGTTAACTTC GATTTGTCGA CTGCCACTGC AAAAACCTAC     120

ACAAAATTTA TCGAAGATTT CAGGGCGACT CTTCCATTTA GCCATAAAGT GTATGATATA     180

CCTCTACTGT ATTCCACTAT TTCCGACTCC AGACGTTTCA TACTCCTCGA TCTTACAAGT     240

TATGCATATG AAACCATCTC GGTGGCCATA GATGTGACGA ACGTTTATGT TGTGGCGTAT     300
```

CGCACCCGCG ATGTATCCTA CTTTTTTAAA GAATCTCCTC CTGAAGCTTA TAACATCCTA   360

TTCAAAGGTA CGCGGAAAAT TACACTGCCA TATACCGGTA ATTATGAAAA TCTTCAAACT   420

GCTGCACACA AAATAAGAGA GAATATTGAT CTTGGACTCC CTGCCTTGAG TAGTGCCATT   480

ACCACATTGT TTTATTACAA TGCCCAATCT GCTCCTTCTG CATTGCTTGT ACTAATCCAG   540

ACGACTGCAG AAGCTGCAAG ATTTAAGTAT ATCGAGCGAC ACGTTGCTAA GTATGTTGCC   600

ACTAACTTTA AGCCAAATCT AGCCATCATA AGCTTGGAAA ATCAATGGTC TGCTCTCTCC   660

AACAAATCTT TTTGGCGCAG AATCAAGGAG GAAAATTTAG AAATCCTGTC GACCTTATAA   720

AACCTACCGG GGAACGGTTT CAAGTAACCA ATGTTGATTC AGATGTTGTA AAAGGTAATA   780

TCAAACTCCT GCTGAACTCC AGAGCTAGCA CTGCTGATGA AAACTTTATC ACAACCATGA   840

CTCTACTTGG GGAATCTGTT GTGAATTGAA AGTTTAATAA TCCACCCATA TCGAAATAAG   900

GCATGTTCAT GAC   913

(2) INFORMATION FOR SEQ ID NO:14:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 32 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTYAARGAYG CNCCNGAYGC NGCNTAYGAR GG   32

(2) INFORMATION FOR SEQ ID NO:15:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 32 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTYTTYATRA TRCANTGNCG NCANCTRGTY CA   32

(2) INFORMATION FOR SEQ ID NO:16:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 32 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGNYTNGAYA CNGTNWSNTT YWSNACNAAR GG   32

(2) INFORMATION FOR SEQ ID NO:17:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGTCTGAACC CGTAACTTGG TAA   23

(2) INFORMATION FOR SEQ ID NO:18:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 21 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CACTCTTCCG TATCTCTCTG T   21

(2) INFORMATION FOR SEQ ID NO:19:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 53 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCAACCCGGG CTAGATACCG TGTCATTCTC AACCAAAGGT GCCACTTATA TTA   53

(2) INFORMATION FOR SEQ ID NO:20:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 23 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTTCATTTTG GCGGCACGTA TCC   23

(2) INFORMATION FOR SEQ ID NO:21:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 46 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTCGAGGCTG CAAGCTTACG TGGGATTTTT TTTTTTTTTT TTTTTT   46

(2) INFORMATION FOR SEQ ID NO:22:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 18 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTCGCTGGAA GGTGAGAA   18

(2) INFORMATION FOR SEQ ID NO:23:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 25 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTCGAGGCTG CAAGCTTACG TGGGA   25

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 35 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGATCTCGAG TACTATTTAG GATCTTTATC GACGA   35

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(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTAAGCAGCA TCTGGAGCAT CT   22

(2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CATTCAAGAA ATTCACGTAG G   21

(2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGCCTGGACA CCGTGAGCTT TAG   23

(2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCGATTGCGA TCCTAAATAG TACTC   25

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 28 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

TTTAGGATCG CAATCGACGA ACTTCAAG   28

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTTCGTCTGT AAAGATCCTA AATAGTACTC GA   32

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGATCTTTAC AGACGAACTT CAAGAGT   27

(2) INFORMATION FOR SEQ ID NO:32:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCTTGTGCTT CGTCGATAAA GATCC   25

(2) INFORMATION FOR SEQ ID NO:33:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 27 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ATCGACGAAG CACAAGAGTG CTATTTT   27

(2) INFORMATION FOR SEQ ID NO:34:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTAAAACCAT GCATAGCACT CTTGAAGTTC GT   32

(2) INFORMATION FOR SEQ ID NO:35:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

AGTGCTATGC ATGGTTTTAC TTGATCAACT GC   32

(2) INFORMATION FOR SEQ ID NO:36:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

AGCACATGTG GTGCCACTTA TATTACCTA   29

(2) INFORMATION FOR SEQ ID NO:37:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 33 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TAAGTGGCAC CACATGTGCT AAAGCTCACG GTG   33

(2) INFORMATION FOR SEQ ID NO:38:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 25 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TGACTGTGGA CAGTTGGCGG AAATA   25

(2) INFORMATION FOR SEQ ID NO:39:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 32 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GCCACTGTCC ACAGTCATTT GAAAGCGCTA CC   32

(2) INFORMATION FOR SEQ ID NO:40:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 36 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GATGATCCTG GAAAGGCTTT CGTTTTGGTA GCGCTT   36

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(2) INFORMATION FOR SEQ ID NO:41:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 41 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

AAGCCTTTCC AGGATCATCA GCTTTTTTGG GCAGCAATGG G   41

(2) INFORMATION FOR SEQ ID NO:42:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 23 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

AAGCCTTTCC AGGATCATCA CAT   23

(2) INFORMATION FOR SEQ ID NO:43:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCGACTCTCT ACTGTTTC   18

(2) INFORMATION FOR SEQ ID NO:44:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CGTTAGCAAT TTAACTGTGA T   21

(2) INFORMATION FOR SEQ ID NO:45:

  (i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 16 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AACAGCTATG ACCATG   16

(2) INFORMATION FOR SEQ ID NO:46:

  (i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 29 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TGAACTCGAG GAAACTACCT ATTTCCCAC   29

(2) INFORMATION FOR SEQ ID NO:47:

  (i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 19 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCATTACATC CATGGCGGC   19

(2) INFORMATION FOR SEQ ID NO:48:

  (i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 64 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: DNA

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GATATCTCGA GTTAACTATT TCCCACCACA CGCATGGAAC AGCTCCAGCG CCTTGGCCAC   60

CGTC   64

(2) INFORMATION FOR SEQ ID NO:49:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 21 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TGTCTGTTCG TGGAGGTGCC G   21

(2) INFORMATION FOR SEQ ID NO:50:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 27 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

CCAAGTGTCT GGAGCTGTTC CATGCGA   27

(2) INFORMATION FOR SEQ ID NO:51:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 29 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GATGTTAAYT TYGAYTTGTC NACDGCTAC   29

(2) INFORMATION FOR SEQ ID NO:52:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 29 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATTGGNAGDG TAGCCCTRAA RTCYTCDAT   29

(2) INFORMATION FOR SEQ ID NO:53:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 32 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GCCACTGCAA AAACCTACAC AAAATTTATT GA   32

(2) INFORMATION FOR SEQ ID NO:54:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 22 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GATGTTAACT TCGATTTGTC GA   22

(2) INFORMATION FOR SEQ ID NO:55:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 33 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TCAACTCGAG GTACTCAATT CACAACAGAT TCC   33

(2) INFORMATION FOR SEQ ID NO:56:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 20 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

    Cys His His His Ala Ser Arg Val Ala Arg Met Ala Ser Asp Glu Phe
    1   5   10   15

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Pro Ser Met Cys
                20

(2) INFORMATION FOR SEQ ID NO:57:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 20 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

        Pro Ser Gly Gln Ala Gly Ala Ala Ala Ser Glu Ser Leu Phe Ile Ser
        1   5   10   15

        Asn His Ala Tyr
                20

(2) INFORMATION FOR SEQ ID NO:58:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 22 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CAGCCATGGA ATCCCATTGC TG   22

(2) INFORMATION FOR SEQ ID NO:59:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 28 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

CACATGTAAA ACAAGACTTC ATTTTGGC   28

(2) INFORMATION FOR SEQ ID NO:60:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 36 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TGAAGTCTTG TTTTAGATGT GTTTTTGAAG AGGCCT   36

(2) INFORMATION FOR SEQ ID NO:61:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 30 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

ATGCCATATG CAATTATAAA CCAACGGAGA   30

(2) INFORMATION FOR SEQ ID NO:62:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 39 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGTTTATAAT TGCATATGGC ATTTTCATCA AGTTTCTTG   39

(2) INFORMATION FOR SEQ ID NO:63:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CTTTCAACAA TGCATTCGCC CGGCGAATAA TAC   33

(2) INFORMATION FOR SEQ ID NO:64:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GCGAATGCAT TGTTGAAAGT TATTTCTAAT TTG   33

(2) INFORMATION FOR SEQ ID NO:65:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GTTTTGTGAG GCAGTTGAAT TGGAAC   26

(2) INFORMATION FOR SEQ ID NO:66:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 34 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TTCAACTGCC TCACAAAACA TTCCATTTGC ACCT   34

(2) INFORMATION FOR SEQ ID NO:67:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 24 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

AAAAGCTGAT GATCCTGGAA AGTG   24

(2) INFORMATION FOR SEQ ID NO:68:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 35 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TCCAGGATCA TCAGCTTTTT TGCGCAGCAA TGGGA   35

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(2) INFORMATION FOR SEQ ID NO:69:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 321 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```
GACATCCAGA TGACTCAGTC TCCATCTTCC ATGTCTGCAT CTCTGGGAGA CAGAGTCACT    60

ATCACTTGCC GGGCGAGTCA GGACATTAAT AGCTATTTAA GCTGGTTCCA GCAGAAACCA   120

GGGAAATCTC CTAAGACCCT GATCTATCGT GCAAACAGAT TGGTAGATGG GGTCCCATCA   180

AGGTTCAGTG GCAGTGGATC TGGGACAGAT TATACTCTCA CCATCAGCAG CCTGCAATAT   240

GAAGATTTTG GAATTTATTA TTGTCAACAG TATGATGAGT CTCCGTGGAC GTTCGGTGGA   300

GGCACCAAGC TTGAAATCAA A                                             321
```

(2) INFORMATION FOR SEQ ID NO:70:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 354 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

```
CAGATCCAGT TGGTGCAGTC TGGACCTGGC CTGAAGAAGC CTGGAGGGTC CGTCAGAATC    60

TCCTGCGCAG CTTCTGGGTA TACCTTCACA AACTATGGAA TGAACTGGGT GAAGCAGGCT   120

CCAGGAAAGG GTTTAAGGTG GATGGGCTGG ATAAACACCC ACACTGGAGA GCCAACATAT   180

GCTGATGACT TCAAGGGACG GTTTACCTTC TCTTTGGACA CGTCTAAGAG CACTGCCTAT   240

TTACAGATCA ACAGCCTCAG AGCCGAGGAC ACGGCTACAT ATTTCTGTAC AAGACGGGGT   300

TACGACTGGT ACTTCGATGT CTGGGGCCAA GGGACCACGG TCACCGTCTC CTCC          354
```

(2) INFORMATION FOR SEQ ID NO:71:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 354 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

```
GACATCCAGT TGGTGCAGTC TGGAGGAGGC CTGGTGAAGC CTGGAGGGTC CGTCAGAATC    60
```

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```
TCCTGCGCAG CTTCTGGGTA TACCTTCACA AACTATGGAA TGAACTGGGT GCGCCAGGCT      120

CCAGGAAAGG GTTTAGAGTG GATGGGCTGG ATAAACACCC ACACTGGAGA GCCAACATAT      180

GCTGATTCTT TCAAGGGACG GTTTACCTTC TCTTTGGACG ATTCTAAGAA CACTGCCTAT      240

TTACAGATCA ACAGCCTCAG AGCCGAGGAC ACGGCTGTGT ATTTCTGTAC AAGACGGGGT      300

TACGACTGGT ACTTCGATGT CTGGGGCCAA GGGACCACGG TCACCGTCTC CTCC            354
```

(2) INFORMATION FOR SEQ ID NO:72:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 321 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```
GACATCCAGA TGACTCAGTC TCCATCTTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACT      60

ATCACTTGCC GGGCGAGTCA GGACATTAAT AGCTATTTAA GCTGGTTCCA GCAGAAACCA      120

GGGAAAGCTC CTAAGACCCT GATCTATCGT GCAAACAGAT TGGAATCTGG GGTCCCATCA      180

AGGTTCAGTG GCAGTGGATC TGGGACAGAT TATACTCTCA CCATCAGCAG CCTGCAATAT      240

GAAGATTTTG GAATTTATTA TTGTCAACAG TATGATGAGT CTCCGTGGAC GTTCGGTGGA      300

GGCACCAAGC TTGAAATCAA A                                               321
```

(2) INFORMATION FOR SEQ ID NO:73:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 70 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```
TGTCATCATC ATGCATCGCG AGTTGCCAGA ATGGCATCTG ATGAGTTTCC TTCTATGTGC      60

GCAAGTACTC                                                            70
```

(2) INFORMATION FOR SEQ ID NO:74:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 78 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

TCGAGAGTAC TTGCGCACAT AGAAGGAAAC TCATCAGATG CCATTCTGGC AACTCGCGAT   60

GCATGATGAT GACATGCA   78

(2) INFORMATION FOR SEQ ID NO:75:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 30 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

TGTTCGGCCG CATGTCATCA TCATGCATCG   30

(2) INFORMATION FOR SEQ ID NO:76:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 15 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

AGTCATGCCC CGCGC   15

(2) INFORMATION FOR SEQ ID NO:77:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TCCCGGCTGT CCTACAGT   18

(2) INFORMATION FOR SEQ ID NO:78:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

TCCAGCCTGT CCAGATGGTG TGTGAGTTTT GTCACAA   37

(2) INFORMATION FOR SEQ ID NO:79:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 76 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CTAACTCGAG AGTACTGTAT GCATGGTTCG AGATGAACAA AGATTCTGAG GCTGCAGCTC   60

CAGCCTGTCC AGATGG   76

(2) INFORMATION FOR SEQ ID NO:80:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

CTAACTCGAG AGTACTGTAT   20

(2) INFORMATION FOR SEQ ID NO:81:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 36 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

TCCAGCCTGT CCAGATGGAC ACTCTCCCCT GTTGAA   36

(2) INFORMATION FOR SEQ ID NO:82:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 18 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GTACAGTGGA AGGTGGAT   18

(2) INFORMATION FOR SEQ ID NO:83:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

CATGCGGCCG ATTTAGGATC TTTATCGACG A   31

(2) INFORMATION FOR SEQ ID NO:84:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

AACATCCAGT TGGTGCAGTC TG   22

(2) INFORMATION FOR SEQ ID NO:85:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 20 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GAGGAGACGG TGACCGTGGT   20

(2) INFORMATION FOR SEQ ID NO:86:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 19 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

GACATCAAGA TGACCCAGT   19

(2) INFORMATION FOR SEQ ID NO:87:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 21 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GTTTGATTTC AAGCTTGGTG C   21

(2) INFORMATION FOR SEQ ID NO:88:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

ACTTCGGCCG CACCATCTGG ACAGGCTGGA G   31

(2) INFORMATION FOR SEQ ID NO:89:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 723 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GACATCCAGA TGACTCAGTC TCCATCTTCC CTGTCTGCAT CTGTAGGAGA CAGAGTCACT   60

ATCACTTGCC GGGCGAGTCA GGACATTAAT AGCTATTTAA GCTGGTTCCA GCAGAAACCA   120

GGGAAAGCTC CTAAGACCCT GATCTATCGT GCAAACAGAT TGGAATCTGG GGTCCCATCA   180

AGGTTCAGTG GCAGTGGATC TGGGACAGAT TATACTCTCA CCATCAGCAG CCTGCAATAT   240

GAAGATTTTG GAATTTATTA TTGTCAACAG TATGATGAGT CTCCGTGGAC GTTCGGTGGA   300

GGCACCAAGC TTGAGATGAA AGGTGGCGGT GGATCTGGTG GAGGTGGGTC CGGAGGTGGA   360

GGATCTGAGA TCCAGTTGGT GCAGTCTGGA GGAGGCCTGG TGAAGCCTGG AGGGTCCGTC   420

AGAATCTCCT GCGCAGCTTC TGGGTATACC TTCACAAACT ATGGAATGAA CTGGGTGCGC   480

CAGGCTCCAG GAAAGGGTTT AGAGTGGATG GGCTGGATAA ACACCCACAC TGGAGAGCCA   540

ACATATGCTG ATTCTTTCAA GGGACGGTTT ACCTTCTCTT TGGACGATTC TAAGAACACT   600

GCCTATTTAC AGATCAACAG CCTCAGAGCC GAGGACACGG CTGTGTATTT CTGTACAAGA   660

CGGGGTTACG ACTGGTACTT CGATGTCTGG GGCCAAGGGA CCACGGTCAC CGTCTCCTCA   720

TGA   723

(2) INFORMATION FOR SEQ ID NO:90:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 723 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GAGATCCAGT TGGTGCAGTC TGGAGGAGGC CTGGTGAAGC CTGGAGGGTC CGTCAGAATC   60

TCCTGCGCAG CTTCTGGGTA TACCTTCACA AACTATGGAA TGAACTGGGT GCGCCAGGCT   120

CCAGGAAAGG GTTTAGAGTG GATGGGCTGG ATAAACACCC ACACTGGAGA GCCAACATAT   180

GCTGATTCTT TCAAGGGACG GTTTACCTTC TCTTTGGACG ATTCTAAGAA CACTGCCTAT   240

TTACAGATCA ACAGCCTCAG AGCCGAGGAC ACGGCTGTGT ATTTCTGTAC AAGACGGGGT   300

TACGACTGGT ACTTCGATGT CTGGGGCCAA GGGACCACGG TCACCGTCTC CTCAGGTGGC   360

GGTGGATCTG GTGGAGGTGG GTCCGGAGGT GGAGGATCTG ACATCCAGAT GACTCAGTCT   420

CCATCTTCCC TGTCTGCATC TGTAGGAGAC AGAGTCACTA TCACTTGCCG GGCGAGTCAG   480

GACATTAATA GCTATTTAAG CTGGTTCCAG CAGAAACCAG GGAAAGCTCC TAAGACCCTG   540

ATCTATCGTG CAAACAGATT GGAATCTGGG GTCCCATCAA GGTTCAGTGG CAGTGGATCT   600

GGGACAGATT ATACTCTCAC CATCAGCAGC CTGCAATATG AAGATTTTGG AATTTATTAT   660

TGTCAACAGT ATGATGAGTC TCCGTGGACG TTCGGTGGAG GCACCAAGCT TGAGATGAAA   720

TGA   723

(2) INFORMATION FOR SEQ ID NO:91:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 51 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CGGACCCACC TCCACCAGAT CCACCGCCAC CTTTCATCTC AAGCTTGGTG C   51

(2) INFORMATION FOR SEQ ID NO:92:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 19 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GACATCCAGA TGACTCAGT   19

(2) INFORMATION FOR SEQ ID NO:93:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 49 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GGTGGAGGTG GGTCCGGAGG TGGAGGATCT GAGATCCAGT TGGTGCAGT .   49

(2) INFORMATION FOR SEQ ID NO:94:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 35 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TGTACTCGAG CCCATCATGA GGAGACGGTG ACCGT   35

(2) INFORMATION FOR SEQ ID NO:95:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 18 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

GACATCCAGA TGACTCAGT

(2) INFORMATION FOR SEQ ID NO:96:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 37 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

TGTACTCGAG CCCATCATTT CATCTCAAGC TTGGTGC   37

(2) INFORMATION FOR SEQ ID NO:97:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

GAGATCCAGT TGGTGCAGTC TG   22

(2) INFORMATION FOR SEQ ID NO:98:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 49 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

CGGACCCACC TCCACCAGAT CCACCGCCAC CTGAGGAGAC GGTGACCGT   49

(2) INFORMATION FOR SEQ ID NO:99:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5                   10                  15
Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20                  25                  30
Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
            35                  40                  45
Lys Ala Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
        50                  55                  60
Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70                  75                  80
Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85                  90                  95
```

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```
Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
            115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
            195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:100:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 251 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: protein

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5               10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
                20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Ala Asp Asp Pro Gly
            35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
        50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
            115             120             125
```

```
Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130                 135             140
Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145                 150             155                 160
Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170                 175
Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190
Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205
Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220
Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240
Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                245             250
```

(2) INFORMATION FOR SEQ ID NO:101:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5               10                  15
Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20                  25              30
Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Ala Asp Asp Pro Gly
        35              40                  45
Lys Ala Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
        50              55              60
Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70              75                  80
Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85              90                  95
Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110
Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125
Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140
Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155                 160
```

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```
Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:102:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5               10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
            165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190
```

```
Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195                 200                 205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
        210                 215                 220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225                 230                 235                 240

Ala Leu Leu Lys Phe Val Cys Lys Asp Pro Lys
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:103:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5                   10                  15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20                  25                  30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35                  40                  45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
        50                  55                  60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70                  75                  80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85                  90                  95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100                 105                 110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115                 120                 125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
        130                 135                 140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145                 150                 155                 160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165                 170                 175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180                 185                 190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195                 200                 205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
        210                 215                 220
```

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```
Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Cys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:104:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5               10                  15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
        100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
    115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
            165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Cys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
            245             250
```

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(2) INFORMATION FOR SEQ ID NO:105:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 251 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5               10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
            20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
            165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Cys Phe Val Asp Lys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:106:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 251 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Cys Gly Ala Thr Tyr Ile Thr
1               5                   10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
                20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
            35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                245             250
```

(2) INFORMATION FOR SEQ ID NO:107:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

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```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5                   10              15

-Tyr-Val-Asn-Phe-Leu-Asn-Glu-Leu-Arg-Val-Lys-Leu-Lys-Pro Glu Gly
                20                  25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
        35              40              45

Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Cys Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70              75                  80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85                  90                  95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
            165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
    195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:108:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
1               5                   10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
                20                  25              30
```

```
     Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
             35                  40                  45

     Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
         50                  55                  60

     Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
     65                  70                  75                  80

     Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                     85                  90                  95

     Gly Leu Phe Lys Asn Thr Cys Lys Thr Arg Leu His Phe Gly Gly Ser
                     100                 105                 110

     Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
             115                 120                 125

     Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
         130                 135                 140

     Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
     145                 150                 155                 160

     Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                     165                 170                 175

     Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
                     180                 185                 190

     Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
             195                 200                 205

     Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
         210                 215                 220

     Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
     225                 230                 235                 240

     Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                     245                 250
```

(2) INFORMATION FOR SEQ ID NO:109:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 251 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

       (ii) MOLECULE TYPE: protein

       (xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

```
     Gly Leu Asp Thr Val Ser Phe Ser Thr Lys Gly Ala Thr Tyr Ile Thr
     1               5                   10                  15

     Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
                     20                  25                  30

     Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Cys Asp Asp Pro Gly
             35                  40                  45

     Lys Cys Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
         50                  55                  60
```

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```
Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65                  70              75                      80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85                  90                      95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
            100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120                 125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150                 155                     160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165                 170                 175

Ile Arg Asn Asn Phe Gln Gln Cys Ile Arg Pro Ala Asn Asn Thr Ile
                180                 185                 190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200                 205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210                 215                 220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230                 235                     240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:110:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Cys Gly Ala Thr Tyr Ile Thr
1               5               10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
                20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Ala Asp Asp Pro Gly
        35              40              45

Lys Ala Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75                      80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
                85              90                      95
```

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```
Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
        100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125

Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130             135             140

Ile Asp Asn Tyr Lys Pro Thr Glu Ile Ala Ser Ser Leu Leu Val Val
145             150             155             160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Asp Lys Asp Pro Lys
                245             250
```

(2) INFORMATION FOR SEQ ID NO:111:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 251 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

```
Gly Leu Asp Thr Val Ser Phe Ser Thr Cys Gly Ala Thr Tyr Ile Thr
1               5               10              15

Tyr Val Asn Phe Leu Asn Glu Leu Arg Val Lys Leu Lys Pro Glu Gly
        20              25              30

Asn Ser His Gly Ile Pro Leu Leu Arg Lys Lys Ala Asp Asp Pro Gly
        35              40              45

Lys Ala Phe Val Leu Val Ala Leu Ser Asn Asp Asn Gly Gln Leu Ala
    50              55              60

Glu Ile Ala Ile Asp Val Thr Ser Val Tyr Val Val Gly Tyr Gln Val
65              70              75              80

Arg Asn Arg Ser Tyr Phe Phe Lys Asp Ala Pro Asp Ala Ala Tyr Glu
            85              90              95

Gly Leu Phe Lys Asn Thr Ile Lys Thr Arg Leu His Phe Gly Gly Ser
        100             105             110

Tyr Pro Ser Leu Glu Gly Glu Lys Ala Tyr Arg Glu Thr Thr Asp Leu
        115             120             125
```

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```
Gly Ile Glu Pro Leu Arg Ile Gly Ile Lys Lys Leu Asp Glu Asn Ala
    130                 135             140

Ile-Asp-Asn-Tyr Lys Pro-Thr-Glu-Ile-Ala-Ser Ser Leu Leu Val Val
145                 150                 155                 160

Ile Gln Met Val Ser Glu Ala Ala Arg Phe Thr Phe Ile Glu Asn Gln
                165             170             175

Ile Arg Asn Asn Phe Gln Gln Arg Ile Arg Pro Ala Asn Asn Thr Ile
            180             185             190

Ser Leu Glu Asn Lys Trp Gly Lys Leu Ser Phe Gln Ile Arg Thr Ser
        195             200             205

Gly Ala Asn Gly Met Phe Ser Glu Ala Val Glu Leu Glu Arg Ala Asn
    210             215             220

Gly Lys Lys Tyr Tyr Val Thr Ala Val Asp Gln Val Lys Pro Lys Ile
225             230             235             240

Ala Leu Leu Lys Phe Val Cys Lys Asp Pro Lys
            245             250
```

(2) INFORMATION FOR SEQ ID NO:112:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

TGATGCGGCC GACATCTCAA GCTTGGTGC   29

(2) INFORMATION FOR SEQ ID NO:113:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 29 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

TGATGCGGCC GACATCTCAA GCTTGGTGC   29

(2) INFORMATION FOR SEQ ID NO:114:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 38 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

TCTAGGTCAC CGTCTCCTCA CCATCTGGAC AGGCTGGA   38

(2) INFORMATION FOR SEQ ID NO:115:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

TTCGAAGCTT GAGATGAAAC CATCTGGACA GGCTGGA   37

(2) INFORMATION FOR SEQ ID NO:116:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 22 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

GAGATCCAGT TGGTGCAGTC TG   22

(2) INFORMATION FOR SEQ ID NO:117:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 98 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

AGTCGTCGAC ACGATGGACA TGAGGACCCC TGCTCAGTTT CTTGGCATCC TCCTACTCTG   60

GTTTCCAGGT ATCAAATGTG ACATCCAGAT GACTCAGT   98

(2) INFORMATION FOR SEQ ID NO:118:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 80 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

TCACTTGCCG GGCGAATCAG GACATTAATA GCTATTTAAG CTGGTTCCAG CAGAAACCAG   60

GGAAAGCTCC TAAGACCCT   80

(2) INFORMATION FOR SEQ ID NO:119:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 80 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

TGACTCGCCC GGCAAGTGAT AGTGACTCTG TCTCCTACAG ATGCAGACAG GGAAGATGGA   60

GACTGAGTCA TCTGGATGTC   80

(2) INFORMATION FOR SEQ ID NO:120:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 79 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

GATCCACTGC CACTGAACCT TGATGGGACC CCAGATTCCA ATCTGTTTGC ACGATAGATC   60

AGGGTCTTAG GAGCTTTCC   79

(2) INFORMATION FOR SEQ ID NO:121:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 82 base pairs
       (B) TYPE: nucleic acid
       (C) STRANDEDNESS: single
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

GGTTCAGTGG CAGTGGATCT GGGACAGATT ATACTCTCAC CATCAGCAGC CTGCAATATG   60

AAGATTTTGG AATTTATTAT TG   82

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(2) INFORMATION FOR SEQ ID NO:122:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 82 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

GTTTGATTTC AAGCTTGGTG CCTCCACCGA ACGTCCACGG AGACTCATCA TACTGTTGAC   60

AATAATAAAT TCCAAAATCT TC

(2) INFORMATION FOR SEQ ID NO:123:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
1   5   10   15

Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Ser Tyr
            20   25   30

Leu Ser Trp Phe His His Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
        35   40   45

Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Tyr
65   70   75   80

Glu Asp Met Gly Ile Tyr Tyr Cys Gln Gln Tyr Asp Glu Ser Pro Trp
            85   90   95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100   105

(2) INFORMATION FOR SEQ ID NO:124:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 118 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1   5   10   15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
        20   25   30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Arg Trp Met
        35   40   45

Gly Trp Ile Asn Thr His Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe
    50   55   60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65   70   75   80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
            85   90   95

Thr Arg Arg Gly Tyr Asp Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr
            100   105   110

Thr Val Thr Val Ser Ser
            115

(2) INFORMATION FOR SEQ ID NO:125:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 107 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Asn Ser Tyr
        20   25   30

Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ala Pro Lys Thr Leu Ile
        35   40   45

Tyr Arg Ala Asn Arg Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Tyr
65   70   75   80

Glu Asp Phe Gly Ile Tyr Tyr Cys Gln Gln Tyr Asp Glu Ser Pro Trp
            85   90   95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100   105

(2) INFORMATION FOR SEQ ID NO:126:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 118 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

```
Glu Ile Gln Leu Val Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1             5               10                  15

Ser Val Arg Ile Ser Cys Ala Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20              25                  30

Gly Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35                  40              45

Gly Trp Ile Asn Thr His Thr Gly Glu Pro Thr Tyr Ala Asp Ser Phe
    50              55                  60

Lys Gly Arg Phe Thr Phe Ser Leu Asp Asp Ser Lys Asn Thr Ala Tyr
65              70                  75                      80

Leu Gln Ile Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Phe Cys
            85                  90                  95

Thr Arg Arg Gly Tyr Asp Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr
            100             105                 110

Thr Val Thr Val Ser Ser
            115
```

(2) INFORMATION FOR SEQ ID NO:127:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 280 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

```
Ala Ala Lys Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala Thr
1             5               10                  15

Phe Asn Val Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala Gly
            20              25                  30

Ile Arg Asn Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg Pro
        35                  40              45

Val Leu Pro Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe His
    50              55                  60

Val Val Leu Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala Ile
65              70                  75                      80
```

```
Arg Ala Asp Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly Thr
                85                  90                  95

Trp Trp Glu Leu Thr Pro Gly Leu Ile Pro Gly Ala Thr Tyr Val Gly
            100                 105                 110

Phe Gly Gly Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu Thr
        115                 120                 125

Asn Val Ala Leu Gly Arg Gln Gln Leu Ala Asp Ala Val Thr Ala Leu
    130                 135                 140

His Gly Arg Thr Lys Ala Asp Lys Ala Ser Gly Pro Lys Gln Gln Gln
145                 150                 155                 160

Ala Arg Glu Ala Val Thr Thr Leu Val Leu Met Val Asn Glu Ala Thr
                165                 170                 175

Arg Phe Gln Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro Lys
            180                 185                 190

Ala Val Glu Lys Lys Ser Gly Lys Ile Gly Asn Glu Met Lys Ala Gln
        195                 200                 205

Val Asn Gly Trp Gln Asp Leu Ser Ala Ala Leu Leu Lys Thr Asp Val
    210                 215                 220

Lys Pro Pro Pro Gly Lys Ser Pro Ala Lys Phe Ala Pro Ile Glu Lys
225                 230                 235                 240

Met Gly Val Arg Thr Ala Glu Gln Ala Ala Asn Thr Leu Gly Ile Leu
                245                 250                 255

Leu Phe Val Glu Val Pro Gly Gly Leu Thr Val Ala Lys Ala Leu Glu
            260                 265                 270

Leu Phe His Ala Cys Gly Gly Lys
            275                 280
```

(2) INFORMATION FOR SEQ ID NO:128:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 280 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

```
Ala Ala Lys Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala Thr
1               5                   10                  15

Phe Asn Val Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala Gly
            20                  25                  30

Ile Arg Asn Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg Pro
        35                  40                  45

Val Leu Pro Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe His
    50                  55                  60

Val Val Leu Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala Ile
65                  70                  75                  80
```

```
Arg Ala Asp Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly Thr
                85                  90                  95

Trp Trp Glu Leu Thr Pro Gly Leu Ile Pro Gly Ala Thr Tyr Val Gly
            100             105             110

Phe Gly Gly Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu Thr
        115             120             125

Asn Val Ala Leu Gly Arg Gln Gln Leu Ala Asp Ala Val Thr Ala Leu
    130             135             140

His Gly Arg Thr Lys Ala Asp Lys Ala Ser Gly Pro Lys Gln Gln Gln
145             150             155                 160

Ala Arg Glu Ala Val Thr Thr Leu Val Leu Met Val Asn Glu Ala Thr
            165             170             175

Arg Phe Gln Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro Lys
            180             185             190

Ala Val Glu Lys Lys Ser Gly Lys Ile Gly Asn Glu Met Lys Ala Gln
        195             200             205

Val Asn Gly Trp Gln Asp Leu Ser Ala Ala Leu Leu Lys Thr Asp Val
    210             215             220

Lys Pro Pro Pro Gly Lys Ser Pro Ala Lys Phe Ala Pro Ile Glu Lys
225             230             235                 240

Met Gly Val Arg Thr Ala Glu Gln Ala Ala Asn Thr Leu Gly Ile Leu
            245             250             255

Leu Phe Val Glu Val Pro Gly Gly Leu Thr Val Ala Lys Cys Leu Glu
            260             265             270

Leu Phe His Ala Ser Gly Gly Lys
        275             280
```

(2) INFORMATION FOR SEQ ID NO:129:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 280 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: protein

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

```
Ala Ala Lys Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala Thr
1               5                   10                  15

Phe Asn Val Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala Gly
            20              25              30

Ile Arg Asn Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg Pro
            35              40              45

Val Leu Pro Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe His
    50              55              60

Val Val Leu Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala Ile
65              70              75                  80
```

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```
Arg Ala Asp Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly Thr
                85                      90                95

Trp Trp Glu Leu Thr Pro Gly Leu Ile Pro Gly Ala Thr Tyr Val Gly
            100                 105                110

Phe Gly Gly Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu Thr
        115                 120             125

Asn Val Ala Leu Gly Arg Gln Gln Leu Ala Asp Ala Val Thr Ala Leu
    130                 135             140

His Gly Arg Thr Lys Ala Asp Lys Ala Ser Gly Pro Lys Gln Gln Gln
145             150             155                     160

Ala Arg Glu Ala Val Thr Thr Leu Val Leu Met Val Asn Glu Ala Thr
            165                 170                 175

Arg Phe Gln Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro Lys
        180             185                 190

Ala Val Glu Lys Lys Ser Gly Lys Ile Gly Asn Glu Met Lys Ala Gln
        195             200             205

Val Asn Gly Trp Gln Asp Leu Ser Ala Ala Leu Leu Lys Thr Asp Val
    210             215             220

Lys Pro Pro Pro Gly Lys Ser Pro Ala Lys Phe Ala Pro Ile Glu Lys
225             230             235                     240

Met Gly Val Arg Thr Ala Glu Gln Ala Ala Asn Thr Leu Gly Ile Cys
            245             250                 255

Leu Phe Val Glu Val Pro Gly Gly Leu Thr Val Ala Lys Ala Leu Glu
            260             265             270

Leu Phe His Ala Ser Gly Gly Lys
            275             280
```

(2) INFORMATION FOR SEQ ID NO:130:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 7 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

```
Ser Cys Asp Lys Thr His Thr
1               5
```

(2) INFORMATION FOR SEQ ID NO:131:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 85 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

TGTCGACATC ATGGCTTGGG TGTGGACCTT GCTATTCCTG ATGGCAGCT GCCCAAAGTG   60

CCCAAGCAGAGATC CAGTTGGTGCA G   82

(2) INFORMATION FOR SEQ ID NO:132:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 86 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

AAGGTATACC CAGAAGCTGC GCAGGAGATT CTGACGGACC CTCCAGGCTT CACCAGGCCT   60

CCTCCAGACT GCACCAACTG GATCTC   86

(2) INFORMATION FOR SEQ ID NO:133:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 84 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

GCAGCTTCTG GGTATACCTT CACAAACTAT GGAATGAACT GGGTGCGCCA GGCTCCAGGA   60

AAGAATTTAG AGTGGATGGG CTGG   84

(2) INFORMATION FOR SEQ ID NO:134:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 85 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

AAAGAGAAGG TAAACCGTCC CTTGAAAGAA TCAGCATATG TTGGCTCTCC AGTGTGGGTG   60

TTTATCCAGC CCATCCACTC TAAAC   85

(2) INFORMATION FOR SEQ ID NO:135:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 87 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

```
GACGGTTTAC CTTCTCTTTG GACGATTCTA AGAACACTGC CTATTTACAG ATCAACAGCC     60

TCAGAGCCGA GGACACGGCT GTGTATT                                         87
```

(2) INFORMATION FOR SEQ ID NO:136:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 92 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

```
GAGGAGACGG TGACCGTGGT CCCTTGGCCC CAGACATCGA AGTACCAGTC GTAACCCCGT     60

CTTGTACAGA AATACACAGC CGTGTCCTCG GC                                   92
```

(2) INFORMATION FOR SEQ ID NO:137:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 84 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

```
GCAGCTTCTG GGTATACCTT CACAAACTAT GGAATGAACT GGGTGAAGCA GGCTCCAGGA     60

AAGGGTTTAA GGTGGATGGG CTGG                                            84
```

(2) INFORMATION FOR SEQ ID NO:138:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 85 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

AAAGAGAAGG TAAACCGTCC CTTGAAGTCA TCAGCATATG TTGGCTCTCC AGTGTGGGTG   60

TTTATCCAGC CCATCCACCT TAAAC   85

(2) INFORMATION FOR SEQ ID NO:139:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 84 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

GACGGTTTAC CTTCTCTTTG GACACGTCTA AGTGCACTGC CTATTTACAG ATCAACAGCC   60

TCAGAGCCGA GGACACGGCT ACAT   84

(2) INFORMATION FOR SEQ ID NO:140:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 91 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: DNA

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

AGGAGACGGT GACCGTGGTC CCTTGGCCCC AGACATCGAA GTACCAGTCG TAACCCCGTC   60

TTGTACAGAA ATATGTAGCC GTGTCCTCGG C   91

(2) INFORMATION FOR SEQ ID NO:141:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 8 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

     Lys Pro Ala Lys Phe Phe Arg Leu
     1   5

(2) INFORMATION FOR SEQ ID NO:142:

      (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 8 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

Lys Pro Ala Lys Phe Leu Arg Leu
1   5

(2) INFORMATION FOR SEQ ID NO:143:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 34 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

GGCCGCAAAG CCGGCTAAGT TCTTMCGTCT GAGT   34

(2) INFORMATION FOR SEQ ID NO:144:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 34 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:
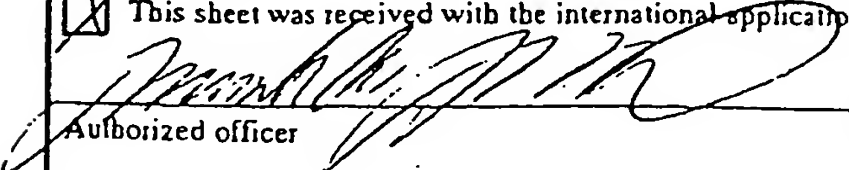
ACTCAGACGK AAGAACTTAG CCGGCTTTGC   30

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page _____ , line _____	See attached sheet

B. IDENTIFICATION OF DEPOSIT   Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection

Address of depositary institution (including postal code and country)

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
See attached sheet	See attached sheet

C. ADDITIONAL INDICATIONS (leave blank if not applicable)   This information is continued on an additional sheet [ ]

"In respect of those designations in which a European patent is sought, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 23(4) EPC)."

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

EPO

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

For receiving Office use only	For International Bureau use only
[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
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ATCC Accession Number	Date Deposited	Page No.
68721	October 2, 1991	4
68722	October 2, 1991	9
69008	June 9, 1992	12
69009	June 9, 1992	18
69101	October 27, 1992	18
69102	October 27, 1992	18
69103	October 27, 1992	18
69104	October 27, 1992	18

CLAIMS

1.   A fusion protein comprising gelonin fused to the amino or carboxy terminal end of an antigen-binding portion of he3 antibody.

2.   The fusion protein of claim 1 wherein said fusion protein lacks a cleavable peptide segment linking said gelonin and said antibody portion.

3.   The fusion protein of claim 1 which is $V_L V_B$-Gel.

4.   The fusion protein of claim 1 which is $V_B V_L$-Gel.

5.   The fusion protein of claim 1 which is Gel-$V_B V_L$.

6.   The fusion protein of claim 1 which is Gel-$V_L V_B$.

7.   The fusion protein of claim 1 which is Gel::kappa,Fd.

8.   The fusion protein of claim 1 which is Gel::Fd,kappa.

9.   A fusion protein comprising gelonin fused to the amino or carboxy terminal end of an antigen-binding portion of an antibody, said fusion protein further comprising the peptide segment CCL between said gelonin and said antibody portion.

10. A fusion protein comprising gelonin fused to the amino or carboxy terminal end of an antigen-binding portion of an antibody, said fusion protein further comprising the peptide segment CCF between said gelonin and said antibody portion.

11. The fusion protein (Gel::RMA::kappa,Fd')$_2$.

12. The fusion protein (Gel::RMA::Fd', kappa)$_2$.

13. The fusion protein Gelonin::RMA::kappa,Fd.

14. The fusion protein Gelonin::RMA::Fd,kappa.

15. The fusion protein Gelonin::SLT::kappa,Fd.

16. The fusion protein Gelonin::SLT::Fd,kappa.

17. The fusion protein $V_H V_L$::SLT::Gelonin.

18. The fusion protein $V_L V_H$::SLT::Gelonin.

19. The fusion protein $V_H V_L$::RMA::Gelonin.

20. The fusion protein $V_L V_H$::RMA::Gelonin.

21. A polynucleotide encoding the fusion protein of claim 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20.

22. A host cell transformed or transfected with the fusion protein of claim 21.

```
RTA       IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50
                                     *
GELONIN   GLD----TVSFSTKGATYITYVNFLNELRVKLKPEGN-SHGIPLLRKKCD   45

RTA       LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFFHPDNQED  100
                                    *
GELONIN   DP-GKCFVLVALSNDNGQLAEIAIDVTSVYVVGYQVRNRSYFF----KDA   90

RTA       AEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISA  150
                                *                       *
GELONIN   PDAAYEGLFKNTIKTRLHFGGSYPSLEG-EKAYRETTDLGIEPLRIGIKK  139

RTA       LYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAP  200
                              * *   **
GELONIN   LDENAIDNYKPTEIASSLLVVIQMVSEAARFTFIENQIRNN--FQQRIRP  187

RTA       DPSVITLENSWGRLSTAIQESN-QGAFASPIQLQRRNGSKFSVYDVSILI  249
                    *
GELONIN   ANNTISLENKWGKLSFQIRTSGANGMFSEAVELERANGKKYYVTAVDQVK  237

RTA       PIIALMVYRCAPPPSSQF  267

GELONIN   PKIALLKFVDKDPK  251
```

FIG. 1

```
RTA   I------FPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPV      44
BRIP  AAKMAKNVDKPLFTATFNVQASSAD-YATFIAGIRNKLRNPAHFSHNRPV      49

RTA   LPN-RVGLPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFF      93
BRIP  LPPVEPNVPPSRWFHVVLKASPTSAGLTLAIRADNIYLEGFKSSDGTWWE      99

RTA   HPDNQEDAEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGP     143
BRIP  LTPGLIPGATYV--------GFGGTYRDLLGDTDKL-TNVALGRQQ         136

RTA   LEEAISALY----YYSTGGTQLPTLARSFIICIQMISEAARFQ----YIE     185
BRIP  LADAVTALHGRTKADKASGPKQQQAREAVTTLVLMVNEATRFQTVSGFVA     186

RTA   GEMRTRIRYNRRSAPDPSVITLENSWGRLSTAIQESNQGAFASPIQLQRR     235
BRIP  GLLHPKAVEKKSGKIGNEMKAQVNGWQDLSAALLKTDVKPPPGKSPAKFA     236

RTA   NGSKFSVYDVSILIPIIALMVYRCAP---------PPSSQF              267
BRIP  PIEKMGVRTAEQAANTLGILLFVEVPGGLTVAKALELFHASGGK          280
```

FIG. 2

```
RTA     IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG    50
                          *
MOMOII  D-------VNFDLSTATAKTYTKFIEDFRATLPFSHKV-YDIPLLYSTIS    42

RTA     LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFFHPDNQED   100
MOMOII  --DSRRFILLDLTSYAYETISVAIDVTNVYVVVAYRTRDVSYFF---KESP    87

RTA     AEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISA   150
                                                        *
MOMOII  PEAYNILFKGTR-KITLPYTGNYENLQTAAHKIRENIDLGLPALSSAITT   136

RTA     LYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAP   200
                                       * *
MOMOII  LFYYNAQSA-----PSALLVLIQTTAEAARFKYIERHVAKYVATNFK--P   179

RTA     DPSVITLENSWGRLSTAI--QESNQGAFASPIQLQRRNGSKFSVYDVS--   246
              *
MOMOII  NLAIISLENQWSALSKQIFLAQNQGGKFRNPVDLIKPTGERFQVTNVDSD   229

RTA     ILIPIIALMVYRCAPPPSSQF                                267

MOMOII  VVKGNIKLLLNSRASTADENFITTMTLLGESVVN                   263
```

FIG. 3

```
RTA     IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50
                  *
LUFFIN  D------VRFSLSGSSSTSYSKFIGDLRKALPSNGTVYNLTILLSSASG    43
                             *

RTA     LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFFHPDNQED  100
LUFFIN  ---ASRYLMTLSNYDGKAITVAVDVSQLYIMGYLVNSTSYFF---NESD    87
                   *

RTA     AEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISA  150
                     *                                  *
LUFFIN  AKLASQYVFKGSTIVTLPYSGNYEKLQTAAGKIREKIPLGFPALDSALTT  137

RTA     LYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAP  200
                              **
LUFFIN  IFHYDSTAA------AAAFLVILQTTAEASRFKYIEGQIIERI--SKNQVP  180
                          *                      * *

RTA     DPSVITLENS-WGRLSTAIQ--ESNQGAFASPIQLQRRNGSKFSVYDVSI  247
LUFFIN  SLATISLENSLWSALSKQIQLAQTNNGTFKTPVVITDDKQQRVEITNVTS  230

RTA     LIPIIALMVYRCAPPPSSQF  267
LUFFIN  KVVTKNIQLLLNYKQNVA    248
```

FIG. 4

RTA   IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50

TRICHO D------VSFRLSGATSSSSYGVFISNLRKALPNERKL-YDIPLL--RSS   40

RTA   LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFFHPDNQED   100

TRICHO LPGSQRYALIHLTNYADETISVAIDVTNVYIMGYRAGDTSYFF--NEASA   88

RTA   AEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISA   150

TRICHO TEAAKYVFKDAMRKVTLPYSGNYERLQTAAGRIRENIPLGLPALDSAITT   138

RTA   LYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAP   200

TRICHO LFYYNANSA-----ASALMVLIQSTSEAARYKFIEQQIGSRV--DKTFLP   181

RTA   DPSVITLENSWGRLSTAIQ--ESNQGAFASPIQLQRRNGSKFSVYDV--S   246

TRICHO SLAIISLENSWSALSKQIQIASTNNGQFESPVVLINAQNQRVTITNVDAG   231

RTA   ILIPIIALMVYRCAPPPSSQF   267

TRICHO VVTSNIALLLNRNNMA   247

FIG. 5

```
RTA    IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG    50
MOMOI  D------VSFRLSGADPRSYGMFIKDLRNALPFREKVYNIPLLLPSVSG     43

RTA    LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSAYFFHPDNQED    100
MOMOI  ---AGRYLLMHLFNYDGKTITVAVDVTNVYIMGYLADTTSYFFNEPAAEL    90

RTA    AEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISA    150
MOMOI  ASQ--YVFRDARRKITLPYSGNYERLQIAAGKPREKIPIGLPALDSAIST    138

RTA    LYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAP    200
MOMOI  LLHYDSTAA-----AGALLVLIQTTAEAARFKYIEQQIQERA--YRDEVP    181

RTA    DPSVITLENSWGRLSTAIQ--ESNQGAFASPIQLORRNGSKFSVYDVSIL    248
MOMOI  SLATISLENSWSGLSKQIQLAQGNNGIFRTPIVLVDNKGNRVQITNVTSK    231

RTA    IPIIALMVY---------RCAPPPSSQF                          267
MOMOI  VVTSNIQLLLNTRNIAEGDNGDVSTTHGFSST                      263
```

FIG. 6

FIG. 7

```
RTA  IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50
                                  *
MAP  A-PTLETIASLDLNNPT--TYLSFITNIRTKVADKTE----QCTIQKIS    42

RTA  LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRA---GNSAYFFHPDN   97
                                *
MAP  KTFTQRYSYIDLIVSSTQKITLAIDMADLYVLGYSDIANNKGRAFFFKDV   92

RTA  QEDAEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEA  147
                               *                      *
MAP  TEAVANNFFPGATGTNRIKLTFGSYGDLEK-NGGLRKDNPLGIFRLENS   141

RTA  ISALYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRR  197
                                       * *
MAP  IVNIY---GKAGDVKKQAKFFLLAIQMVSEAARFKYI-SDKIPSEKYEE-  186

RTA  SAPDPSVITLENSWGRLSTAIQESNQGAFASPIQLQRRNGSKFSVYDVSI  247
                        *
MAP  VTVDEYMTALENNWAKLSTAVYNSKPSTTATKCQLATSPVTISPWIFKT   236

RTA  LIPIIALMVYRCAPPPSSQF  267

MAP  VEEIKLVMGLLKSS        250
```

FIG. 8

RTA   IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50
PAPS   I-----NTITFBAGNATINKYATFHESLRNEAKDPSLKCYGIPHLPNTNS   45

RTA   LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNSA-----YFFHP   95
PAPS   --TIKYLLVKLQGASLKTITLHLRRNNLYVHGYSDPYDNKCRYHIFNDI   92

RTA   DNQEDAEAITHLFTDVQNRYT--FAFGGNYDRLEQLAG-NLRENIELGNG   142
PAPS   KGTEYSDVENTLCIPSSNPRVAKPINYNGLYPTLEKKAGVTSRNEVQLGIQ   142

RTA   PLEEAISALYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRTRI   192
PAPS   ILSSDIGKI--SGQGSFTEKIEAKFLLVAIQMVSEAARFKYIENQVKTN-   189

RTA   RYNRRSAPDPSVITLENSWGRLSTAIQESNQGAFASPIQLQRRNGSKFSV   242
PAPS   -FNRDFSPNDKVLDLEENWGKISTAIHNSKNGALPKPLELKNADGTKWIV   238

RTA   YDVSILIPIIALMVYRCAPPPSSQF   267
PAPS   LRVDEIKPDVGLLNY--VNGTCQAT   261

```
RTA    IFPKQYPIINFTTAGATVQSYTNFIRAVRGRLTTGADVRHEIPVLPNRVG   50
SAP6   V-----TSITLDLVNPTAQQYSSFVDKIRNNVKDPNLKYGGTDI--AVIG   43

RTA    LPINQRFILVELSNHAELSVTLALDVTNAYVVGYRAGNS-----AYFFHP   95
SAP6   PPSKEKFLRINFQSSRG-TVSLGLKRDNLYVVAYLAWDNTNVNRAYYFRS   92

RTA    DNQEDAEAITHLFTDVQNRYTFAFGGNYDRLEQ------LAGNLRENIELG  140
SAP6   EITSAESTALFPEATTANQKALEYTEDYQSIEKNAQITQGDQSRKELGLG  142

RTA    NGPLEEAISALYYYSTGGTQLPTLARSFIICIQMISEAARFQYIEGEMRT  190
SAP6   IDLLSTSHEAV---NKKARVVKDEARFLLIAIQMTAEAARFRYIQNLVIK  189

RTA    RIRYNRRSAPDPSVITLENSWGRLSTAI-QESNQGAFASPIQLQRRNGSK  239
SAP6   N--FPNKFNSENKVIQFEVNWKISTAIYGDAKNGVFNKDYDFGFGKVRQ  237

RTA    FSVYDVSILIPIIALWVYRCAPPPSSQF  267
SAP6   VKDLQWGLL------MYLGKPKSSNEAN  259
```

FIG. 9

```
pos          10        20        30              40                5C
bind   +-+++++  o++++++o++-+-++----+--  --  ----ooo+++o+++--+----   ---
bury   +-+-+-+  o++o+-+++++-+--+-+++  -+  +-----=o-+++-ro-+---=    +--
mod    .  .  .  ..  .  .  ...  .  -  .  .          .  ..    .
hK1    DIQMTQS  PSSLSASVGDRVTITCrASQx Is   xyLxWYQQKPGkAPkiLIY       aAS
hK3    EIVLTQS  PgTLSlSPGERATLSCRASQS vs   ssyLAWYQQKPGQAPRLLIY      gaS
hK2    DIVMTQS  PLSLPVTPGZPASISCRSSQS Ll   nnYLnWYLGKPGQSPQLLIY      igS
hL1    xSVLTQP  PS aSgtPGQrVtISCsGssS iG   xnxVxWYQqlPGtAPkLLIY      n n
hL2    xSALTQP  aS VSGSPGQSiTISCtGtss Vg   ynxVSWyQhPGkAPK LIy       dv
hL3    SYeLTQP  PS vSVsPGQTA ITCsGdx  lx   xxyvxWYQQkPgQaPviVIY      d
hL6    nfmltqp  ns vsespgktvtisctxsxg ia   sxyvqwyqqrpgsapttviy .    edn
hK4    divmtqs  pdslavslgeratinckssqs vl   knylawyqqkpgqppxlliy      was
hL4    seltqp   ps vsvapgqt ritcsgdx  ig   xydaxwyqqkpgqapllviy      grn
hL5    saltqp   ps asgspgqsvtisctgtss vg   xxyvswyqqh g apk ·l       ev

pos        60        70            80        90            100
bind   -+oo++o+++-++--++  -++++++++++-++++++---o---------  -o o++++++-----
bury   ++o++-+o+o-+-+o+o++  -+-+-+++-++-=+--=-=-=+=--=  ==  =-+=-+--+++
mod    .  .  .  .  ..     .  .  ..  ...  .      .  .  ....
hK1    xLxsGVPSRFsGSGSGTx  FTlTISsLQpeDfATYYCqqyxxxP    xt  FGqGTkv eik
hK3    sRATGIPdRFSGSGSGTD  FTLTISrLEPGDFAVYYCQQYgssP    xT  FGQGTKv EIK
hK2    nRaSGVPDRFSGSGSGTD  FTLXISRVeAEDVGVYYCMQalqxP    xT  FGQGTkx EIK
hL1    RPSGvPDRFSgSKSGTS   hsLaIsGLqseDeaDYYCatWDdSLd   pV  FGGGTk TVLq
hL2    RPSGv  RFSGSKSGnT   ASLTISGiLQaeDEAdYYCSSYxgxxx  xV  FGgGtkltVLg
hL3    RPSgIPQRFSGS St T   ATLTISGvqa DEADYYCqxwDxxx    vv  FGGGTkLTVLg
hL6    rpsgvpdrfsgsss ns   asltisglktedeadyycqsydsxx    wv  fgggtkltvig
hK4    resgvpdrfsgsgsgtd   ftltisslqaqdvavyycqqyystp    xt  fgqgtkx gik
hL4    rpsgipdrfsgsssght   asltitgaqaedeadyycnsrdssgk   vl  fgggtkltvig
hL5    rpsgvpdrfsgsks nt   asltvsgl aedeadyycssyxxxxx   fv  fg gtk tvl
```

FIG. 10A

```
pos           10            20        30          40          50
bind  o-+o+++o+    +++o++++++-+oo-----  ----ooo++ro++r-o-oo---------
bury  +-+-+-++o+   +o+o+++-+-+-++-+-o+-+  +o+-+--o-+++ro-o--o--oo++o++
mod    • . •• .     • . ••• • • •              • ••
hH3   EVQLvESGGG   LVqPGGSLRLSCAASGFtFs  xxxmxWVRQApGKgLEWVxx1xxxxxgx
hH1   QVqLvqSGaE   VxKPGxSvxvSCKxSGyyFx  xyxixWvRQaPGxGLEWvGxixpxxcxt
hH2   xvtlxesgpx   lvlptqtltltctvsgxsls  xxxvxwirqppgkxlewiaxix  xadd

pos    60       70        80          90        100        1.0
bind  -oooo+o+o+ io+-+o++o+o++++++++++-+-++--o-----------o+++-+-+-++
bury  =o=+o-++o-+--o+o++o+---+-+-++---+r--+-o---=--=-ooooo=--=---+or---+
mod    •  • . . •• .. •   • • •• ••• •:        • . . ••
hH3   xyadSVkGRFTISRddSKNtlyLqMnsLraeDTAvYYCarxxxxxxxxxxxWGqGTlVTvSS
hH1   xyapxfqgRVTxtrdxSxntayMeLxsLrseDtAvYYCArxxxxxxxxxxxwgqGtlvtVSS
hH2   xyxtslrsrltiskdtsknqvvlxxxxxdpxdtatyycarxxxxxxxxxdvvgqgttvtvss
```

FIG. 10B

SH65K-1
AGT CGT CGA CAC GAT GGA CAT GAG GAC CCC TGC TCA GTT TCT TGG
CAT CCT CCT ACT CTG GTT TCC AGG TAT CAA ATG TGA CAT CCA GAT
GAC TCA GT

HUH-K1
TGA CTC GCC CGG CAA GTG ATA GTG ACT CTG TCT CCC AGA CAT GCA
GAC ATG GAA GAT GAG GAC TGA GTC ATC TGG ATG TC

HUH-K2
TCA CTT GCC GGG CGA GTC AGG ACA TTA ATA GCT ATT TAA GCT GGT
TCC AGC AGA AAC CAG GGA AAT CTC CTA AGA CCC T

HUH-K3
GAT CCA CTG CCA CTG AAC CTT GAT GGG ACC CCA TCT ACC AAT CTG
TTT GCA CGA TAG ATC AGG GTC TTA GGA GAT TTC C

HUH-K4
GGT TCA GTG GCA GTG GAT CTG GGA CAG ATT ATA CTC TCA CCA TCA
GCA GCC TGC AAT ATG AAG ATT TTG GAA TTT ATT ATT G

HUH-K5
GTT TGA TTT CAA GCT TGG TGC CTC CAC CGA ACG TCC ACG GAG ACT
CAT CAT ACT GTT GAC AAT AAT AAA TTC CAA AAT CTT C

HUH-G1
TGT CGA CAT CAT GGC TTG GGT GTG GAC CTT GCT ATT CCT GAT GGC
AGC TGC CCA AAG TGC CCA AGC ACA GAT CCA GTT GGT GCA G

HUH-G2
AAG GTA TAC CCA GAA GCT GCG CAG GAG ATT CTG ACG GAC
CCT CCA GGC TTC TTC AGG CCA GGT CCA GAC TGC ACC AAC TGG ATC T

HUH-G3
GCA GCT TCT GGG TAT ACC TTC ACA AAC TAT GGA ATG AAC TGG GTG
AAG CAG GCT CCA GGA AAG GGT TTA AGG TGG ATG GGC TGG

HUH-G4
AAA GAG AAG GTA AAC CGT CCC TTG AAG TC A TCA GCA TAT GTT GGC
TCT CCA GTG TGG GTG TTT ATC CAG CCC ATC CAC CTT AAA C

HUH-G5
GAC GGT TTA CCT TCT CTT TGG ACA CGT CTA AGT GCA CTG CCT ATT
TAC AGA TCA ACA GCCTCA GAG CCG AGG ACA CGG CTA CAT

HUH-G6
AGG AGA CGG TGA CCG TGG TCC CTT GGC CCC AGA CAT CGA AGT ACC
AGT CGT AAC CCC GTC TTG TAC AGA AAT ATG TAG CCG TGT CCT CGG C

H65G-2S
ACT AGT GTC GAC ATC ATG GCT TGG GT

H65-G2
GAG GAG ACG GTG ACC GTG GT

H65K-2S
AGT CGT CGA CAC GAT GGA CAT GAG GAC

FIG. 11

JK1-HindIII
GTT TGA TTT CAA GCT TGG TGC

```
pos                10        20        30        40        50
EU    DIQMTQS PSTLSASVGDRVTITCRASQS IN  TWLAWYQQKPGKAPKLLMY   KAS
hK1   DIQMTQS PSSLSASVGDRVTITCrASQx Is  xyLxWYQQKPGKAPklLIY   aAS
TAC   QIVLTQS PAIMSASPGEKVTITCSASSS IS  YMHWFQQKPGTSPKLWIY   TTS
bind  +-+++++ o+++++++o+++++++-+--  --  ---ooo++++o+++-+---   ---
bury  +-+-+-+ o++o+-++++-+-+-++-++++ -+  ++-=-=o=+++++o+=--=   +++
mod    • •  •  • ••  • •  •• • • • • •                • •• •
M/H   H HM    HHM   M HH    h   M    M MM M    hM   M    MM
prop  DIQLTQS PSSMSASPGDRVTITCRASSS IS  YMHWFQQKPGKSPKLWIY   TTS
Que   DIQMTQS PSTLSASVGDRVTITCSASSS IS  YMHWYQQKPGKAPKLLIY   TTS
```

```
pos            60        70        80        90        100
EU    SLESGVPSRFIGSGSGTE   FTLTISSLQPDDFATYYCQQYNSDS   KM FGQGTKV EVK
hK1   xLxsGVPSRFsGSGSGTx   FT1TISsLQpeDfATYYCqqyxxxP   xt FGqGTkv eik
TAC   NLASGVPARFSGSGSGTS   YSLTISRMEAEDAATYYCHQRSTYP   LT FGSGTKL ELK
bind  -+oo++o+++++-++--++   -+++++++++++++++-o------   -o o++++++ +++
bury  ++o++-+o-+-+o+o++   -+-+-++-+++-+-=-=-=-=+-==   == =-+=-+- +++
mod    •  •  •  •  •   ••   •  •  ••  •••  •            •   •  •  •••
M/H   M M     H         m   MH    hMHm  h   M MMMM    M    h   M  m
prop  NLASGVPSRFSGSGSGTS   YTLTISSMQAEDFATYYCHQRSTYP   LT FGQGTKL ELK
Que   NLASGVPARFSGSGSGTE   FTLTISSLQPDDFATYYCHQRSTYP   LT FGQGTKV EVK
```

FIG. 12A

```
pos                10              20          30        40              50
EU      CVQLVQSGAE    VKKPGSSVKVSCKASGGTFS  RSAIIWVRQAPGQGLEWMGGIVPMFGPP
hH1     CVqLvqSGaE    VkKPGxSvxvSCKxSGyyFx  xyxixWvRQaPGxGLEWvGxixpxxgxt
TAC     CVQLQQSGAE    LAKPGASVKMSCKASGYTFT  SYRMHWVKQRPGQGLEWIGYINPSTGYT
bind    o--o+--o+     +++o+++--+-+oo----    ----ooo++o++-o-oo---------
bury    +-+---++o+     +o+o++++-+-+--+-o+-+  +o+-=--o=+++++o=o=-o--oo++o++
mod      . . .. .      . . .... . .           . ..
M/H         M         hM   m  mM    M    M M M MMM  M h   m    M M M MM M
prop    QVQLQQSGAE    VAKPGASVKMSCKASGYTFT  SYRMHWVKQAPGQGLEWIGYINPSTGYT
Que     QVQLVQSGAE    VKKPGSSVKVSCKASGYTFT  SYRMHWVRQAPGQGLEWIGYINPSTGYT


pos     60       70        80         90         100        110
EU      NYAQKFQGRVTITADESTNTAYMELSSLRSEDTAFYFCAG GYGIY SPEEYNGGLVTVSS
hH1     xyapxfqgRVTxtrdxSxntayMeLxsLrseDtAvYYCArxxxxxxxxxxwgqGtlvtVSS
TAC     EYNQKFKDKATLTADKSSSTAYMQLSSLTFEDSAVYYCAR*GGGV  FDYWGQGTTLTVSS
bind    -oooo+o+o++o+--+o++o+o++++++++++++++++++-o--------o+++++++-++
bury    =o=+o-++o-+--+o+o++o+-+-+-++-+++-+-o-=---==ooooo==-==-=o+---++
mod      . . . . .. . . ...  . .... .                . ..
M/H     M MMM MhMM M M M mM    h m hh  h         MMMM  MMM      M
prop    EYNQKFKGKATLTADKSSSTAYMELSSLRSEDTAVYYCAR GGGV  FDYWGQGTTLTVSS
Que     EYNQKFKDKATITADESTNTAYMELSSLRSEDTAVYYCAR GGGV  FDYWGQGTLVTVSS
```

FIG. 12B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5   C12N15/62   C07K15/12   C12N1/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5   C12N   C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 22324 (XOMA CORPORATION) 23 December 1992 cited in the application see Examples 3 and 7 and claims.	1,9
A	PROC. NATL. ACAD. SCI. USA vol. 90 , 1993 pages 457 - 461 M. BETTER ET AL.; 'Potent ant-CD5 ricin A immunoconjugates from bacterially produced Fab' and F(ab')2' cited in the application see the whole document.	1,9
A	EP,A,0 506 124 (TANOX BIOSYSTEMS, INC.) 30 September 1992 see column 4, line 31 - column 6, line 11 and claims.	1-8

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family.

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 1994	2 1. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Yeats, S

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 05168 (RESEARCH DEVELOPMENT FOUNDATION) 18 March 1993 see claims. ---	1,9
P,X	WO,A,93 09130 (XOMA CORPORATION) 13 May 1993 cited in the application see Examples 10 and 12. ---	1,2, 13-16
P,X	J. BIOL. CHEM. vol. 269 , 1994 pages 9644 - 9650 M. BETTER ET AL.; 'Gelonin analogs with engineered cysteine residues form antibody immunoconjugates with unique properties' see abstract and Table IV. -----	1

1

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		EP-A-	0602144	22-06-94
		FI-A-	941048	04-03-94
WO-A-9309130	13-05-93	CA-A-	2122714	13-05-93